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The Antibacterial Effect of Nickel Nanoparticles Against *Streptococcus mutans* Compared to Chlorhexidine

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Abstract

Background & Objectives: Due to the increasing trend of extensive antibiotic resistance among bacterial strains and side effects, seeking novel methods such as nanoparticles (NPs) is promising for infection eradication.

Materials & Methods: Eighteen *Streptococcus mutans (S. mutans)* clinical isolates were collected from dental plaques. Moreover, *S. mutans* ATCC25175 standard strain was obtained from Pasteur institute of Iran. Following preparation of nanoparticles, their antibacterial effects were assessed compared to chlorhexidine. The nickel NPs (Ni-NPs) was prepared and its antibacterial effect was compared to the 12% chlorhexidine. The minimum inhibitory and bactericidal concentrations (MIC and MBC, respectively) of Ni-NP (dilution range: 0.125-64µg/mL) were measured using broth microdilution method.

Results: The nickel NPs (Ni-NPs) was prepared and its antibacterial effect was compared to the 12% chlorhexidine. The minimum inhibitory and bactericidal concentrations (MIC and MBC, respectively) of Ni-NP (dilution range: 0.125-64µg/mL) were measured using broth microdilution method. The MIC and MBC levels of Ni-NP against the clinical isolates ranged 2-16µg/mL and 4-16µg/mL, respectively. These values against the *S. mutans* ATCC27175 standard strain included 4 and 8µg/mL, respectively. Furthermore, the MIC and MBC of chlorhexidine against clinical isolates ranged 8-64 and 32-64µg/mL, respectively, while both included 64µg/mL against standard strain ($p<0.001$).

Conclusions: The results of this study outlined that Ni-NPs exert efficient antibacterial effect at nontoxic concentrations compared to 12% chlorhexidine.

Keywords: Dental caries, *Streptococcus mutans*, Nickel nanoparticles, Chlorhexidine, antibacterial effect

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Introduction

Dental caries and periodontal diseases are caused by the accumulation of microorganisms and the formation of microbial plaque (1). Therefore, the necessary measures to reduce or inhibit the accumulation of microbial plaque lead to the control of these complications. *Streptococcus mutans (S. mutans)* strains are Gram-positive cocci, facultative anaerobic and catalase-negative bacteria which cause dental caries due to dental plaque (2, 3). Periodontitis is a chronic infection

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of the gums that affects the hard and soft tissue around the teeth. Progression of the disease leads to the destruction of collagen fibers around the tooth and the degeneration of the alveolar bone in that area. One of the main mechanisms of dental plaque formation is the formation of biofilm, in which bacterial species attach to a living or non-living surface and are enclosed in an extracellular layer (4-6). The formation of biofilms protects the bacteria against the immune system and antimicrobial agents, resulting in significant increase in the bacterial resistance. Biofilm formation is a pathogenicity mechanism employed by *S. mutans.* On the other hand, excessive use of antibiotics, in addition to side effects, leads to the development of this type of resistance and limitation of treatment options (7-10). Therefore, seeking novel antimicrobial compounds with low side effects and lack of (or rare) induction of bacterial resistance mechanisms seems essential. Currently, nanoparticles (NPs) have arrived as novel alternatives of antibiotics in this regard. Metal nanoparticles show different antibacterial properties based on surface to volume ratio. Antimicrobial NPs have advantages over conventional antibiotics, such as lower host toxicity, resistance induction, lower cost, and longer duration of action in the body (11, 12). Ni-NPs are spherical black particles providing a huge amount of surface exerting antibacterial effects depending on the concentration and the time of exposure. Owing to the lower toxicity levels of metal NPs compared to those of metal ions, these compounds can be evaluated in laboratory animals *(in vivo)* and patients body (clinical trial) models and have lasting effects (10-13). Several previous studies have revealed that the Nano-silver (Ag), Nano-nickel, Nano-titanium, copper (Cu), bismuth and Nano-gold (Au) compounds have conferred antibacterial effects against both Gram-positive and Gram-negative bacteria (11-15). Meanwhile, Nano-nickel compounds had antibacterial and anti-biofilm effects on *Escherichia coli, Staphylococcus aureus, S. mutans, Bacillus cereus, Klebsiella pneumoniae,* and *Candida albicans*, which were obtained from clinical and food origins (11-25). These effects were dependent on the bacterial species or different strains, NP concentration and exposure time. However, the method of NP synthesis and the antimicrobial testing route also affect the accuracy of the results. In a study, nickel titanium with size of 2 to 16 nm had antibacterial effects against *S. mutans* (26). The aim of our study was to evaluate the effect of Ni-NPs against *S. mutans* clinical strains compared to chlorhexidine by MIC method.

Materials and Methods

Bacterial culture

Eighteen *S. mutans* clinical isolates were collected from dental plaques. Moreover*, S. mutans* ATCC25175 standard strain was obtained from Pasteur institute of Iran and kept in trypticase soy broth (TSB). The strains were cultured onto the blood agar medium for further studies.

Preparation of suspension containing nanoparticle

Briefly, 10mg of NP powder was dissolved into 200mL ddH$_2$O under sonication at 10W for 21 min onto the ice to prepare a stock solution. The solution was filtered using a 0.2 micron filter and the final concentration was obtained using spectrophotometer (11, 13).

DNA extraction

The total DNA was extracted using the boiling method. Briefly, following bacterial culture onto the nutrient agar, two colonies were taken and suspended into the 200µL of ddH$_2$O. Next, the tubes were boiled for 15min. Then, the tubes were centrifuged at 10,000 RPM for 10 minutes and the supernatant containing the DNA was taken. The DNA purity was measured using the spectrophotometer at optical density (OD) 260/280nm ratio to be >1.8µg/mL.

Polymerase Chain Reaction

The polymerase chain reaction (PCR) was

performed for the identification of *S. mutans* (primer F: 5’-GCACCACAACATTGGGAAGCTCAGTT-3’ and R: 5’-GAATGGCCGCTAAGTCAACAGGAT-3’) and *S. salivarius* (F: 5’-GTGTTGCCACATCTTCACTCGCTTCGG-3’ and R: 5’–CGTTGATGTGCTTGAAAGGGCACCATT-3’) strains. In a total volume of 25µL, master mix, ddH_2O and template DNA were mixed and placed into the thermal cycler. The annealing temperature of *S. mutans* and *S. salivarius* specific genes included 53˚C and 55˚C, respectively which amplified 433 and 544bp products, respectively. The products were visualized using electrophoresis and ethidium bromide dye (14-16). In a total volume of 25µL, master mix, ddH_2O and template DNA were mixed and placed into the thermal cycler. The annealing temperature of *S. mutans* and *S. salivarius* specific genes included 53˚C and 55˚C, respectively which amplified 433 and 544bp products, respectively. The products were visualized using electrophoresis and ethidium bromide dye (14-16).

MIC and MBC determination

The minimum inhibitory and bactericidal concentrations (MIC and MBC, respectively) were determined using broth dilution method. The range of NP concentrations included 0.125-64µg/mL. The concentration was serially diluted and added to the bacterial suspension which was equal to the MC Farland standard turbidity. The test was performed in duplicate and the tubes were incubated at 37°C for 24h. The lowest concentration without bacterial growth was considered as the MIC. For MBC determination, 100µL of suspensions without bacterial growth was cultured onto the Mueller Hinton agar (Merk, Germany) and incubated for 24h. The colonies were counted and each concentration conferring 9.99% of bacterial growth inhibition was considered as MBC.

Data analysis

The data were analyzed using SPSS version 22. The t-test was used for comparison between groups at a significance level of 0.05.

Results

Bacterial isolates

Eighteen *S. mutans* clinical isolates and the *S. mutans* ATCC27175 standard strain were included (Figures 1 and 2).

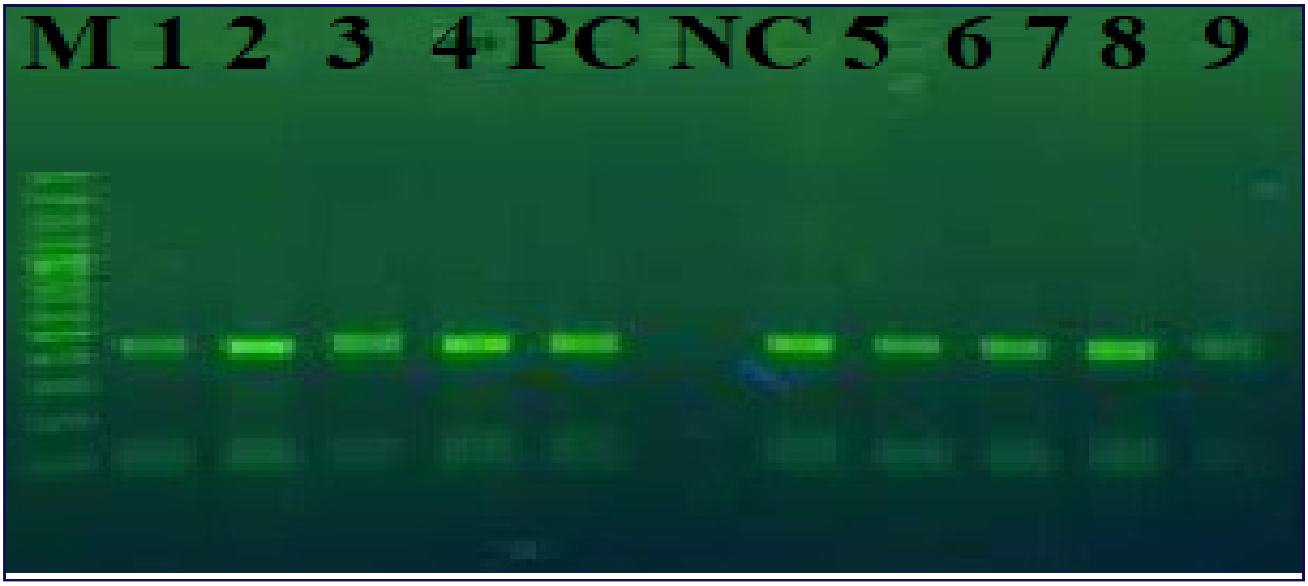

Figure 1. Gel electrophoresis of PCR products of the *S. mutans* specific gene (433bp); M: 100bp DNA marker, 1-4 and 6-9: positive samples, PC: positive control, NC: negative control

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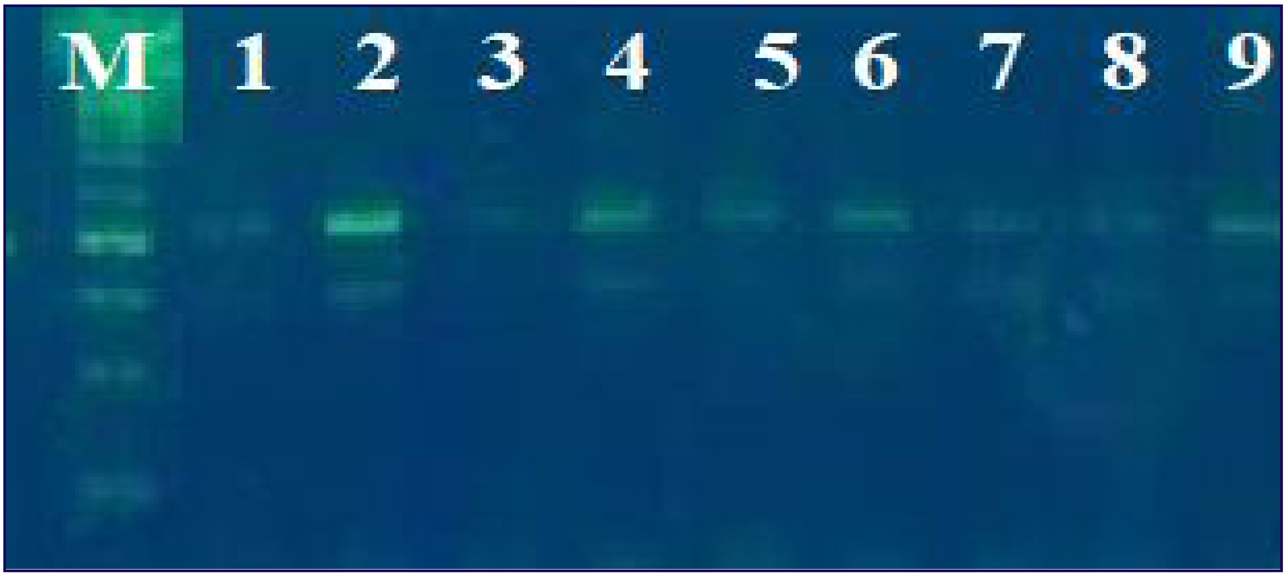

Figure 2. Gel electrophoresis of PCR products of the *S. salivarius* specific gene (544bp); M: 100bp DNA marker, 1-9: positive samples

The MIC and MBC levels

The MIC and MBC levels of NiNP against the clinical isolates respectively ranged 2-16µg/mL and 4-16µg/mL (Table 1). These values against the *S. mutans* ATCC27175 standard strain included 4 and 8µg/mL, respectively. Furthermore, both the MIC and MBC of chlorhexidine against clinical isolates ranged 32-64µg/mL, while including 64µg/mL against standard strain (p<0.001).

Table1. The comparison of MIC and MBC values of Ni-NP and chlorhexidine against clinical isolates of *S. mutans*

Isolate	Ni-NP MIC (µg/mL)	Ni-NP MBC (µg/mL)	Chlorhexidine MIC (µg/mL)	Chlorhexidine MBC (µg/mL)	p value
1	4	8	16	64	<0.001
2	8	16	32	64	<0.001
3	4	8	32	64	<0.0001
4	4	16	16	32	
5	8	16	16	32	
6	2	4	32	64	

7	4	8	16	32	<0.001
8	8	8	8	16	0.0011
9	4	8	8	32	<0.0001
10	16	32	16	32	0.889
11	4	8	32	64	<0.0001
12	4	8	16	32	<0.0001
13	4	8	16	64	<0.0001
14	8	16	32	64	<0.001
15	4	8	8	16	<0.001
16	2	4	16	32	<0.0001
17	4	8	16	32	<0.0001
18	8	16	16	64	<0.0001
19	8	8	32	64	<0.0001

MIC: minimum inhibitory concentration, MBC: minimum bactericidal concentration, Ni-NP: nickel nanoparticle

As depicted in Table 1, the MIC and MBC values of Ni-NP were significantly lower than those of chlorhexidine, except for one isolate.

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Discussion

In our study, the Ni-NPs MIC and MBC values were significantly lower than those of chlorhexidine. These findings were due to the higher surface access by the Ni-NPs which affects the cell wall of the bacterial species. Owing to the advent and development of antibiotic-resistant bacterial pathogens, various NPs have been synthesized and investigated for this aim. NiNPs have lower cost compared to the Ag NPs to be used in dentistry (12,14). In this study, the NiNP conferred significantly higher (2-16µg/mL) antibacterial effect than that of 12% chlorhexidine (8-64µg/mL) against *S. mutans.* In other words, NiNP inhibited the isolates at lower concentrations than 12% chlorhexidine. However, the results of some studies have exhibited that chlorhexidine has stronger antibacterial effects on dental plaque compared to those from other agents. A number of studies have also revealed the antimicrobial and anti-biofilm effects of metal NPs against microorganisms (11-25). Jose et al., demonstrated that Schiff based ligand-NiNPs 50% cell cytotoxicity was >300µg/mL and it had dose-dependent bacterial killing effect against a number of nosocomial pathogens (19). In another study by Ahghari et al., nickel magnetic mirror nanoparticles (NMMNPs) exerted substantial antibacterial (80%) activities against both *E. coli* and *S. aureus* at 100mg for 18-24h of exposure (18). In addition, the antibacterial effects of nickel and titanium NPs were shown against *E. coli* clinical isolates with low cell cytotoxicity than metal ions and could be administered to living organisms and had a long-lasting effect (13).

We observed that the antimicrobial effect of NiNP was depended on the concentration (2-16µg/mL) and time of exposure. Interestingly, the MIC and MBC values of NiNP in this study were considerably lower than those from other studies which were possibly due to the different bacterial species (14-18). It is notable that MIC and MBC levels have not been determined in previous studies and the zone of growth inhibition onto the agar medium has been detected in spite of the superiority of broth microdilution (27). For instance, Helen et al. observed that NiNP growth inhibitory zone diameter against *S. aureus*, *E. coli*, *B. cereus* and *K. pneumoniae* included 14, 13, 10 and 9 mm, respectively (14). It was demonstrated that NiNP had strong antimicrobial effects against *S. aureus* and *S. mutans* with the MIC of 1000µg / mL. Different MIC levels with those of our results were possibly due to differences in the NPs synthesis method which has an important effect on the properties of NPs, such as antimicrobial traits (27).

It was exhibited that NiNPs had the ability to inhibit biofilm formation by mupirocin-resistant *S. aureus* (23) at a concentration of 11 mg /mL, and similar to our results, had a strong antimicrobial effect. Indeed, *S. aureus* has been more resistant than *S. mutans* in exposure to NiNPs. NiNP at concentration of 1 µg/mL had a significant biofilm inhibitory effect against *S. mutans* (24).

A study demonstrated that AuNP and NiNPs MIC levels against *S. aureus* isolated from milk included 0.42 and 0. 21 mg/mL, respectively. In addition, the AuNP and NiNP MIC values against *E. coli* included 0.84 and 0.21 mg/mL, respectively (16). A study by Khashan et al., the antimicrobial effect of colloidal NiNP was exhibited against *P. aeruginosa*, *E. coli*, *S. aureus* and *S. pneumoniae* using broth micro-dilution method at concentrations between 400 and 1000 µg/mL. Although previous findings alongside our results have demonstrated the antibacterial effects of NiNP against clinical isolates of *S. mutans*, validation of NiNP sensitivity, synthetic approaches and future in vivo studies will help to fill the gap of NPs protection levels to apply in formulations and understanding mechanisms of action.

Conclusion

NiNPs outlined substantial antibacterial effect which was significantly higher than that of 12% chlorhexidine against *S. mutans.* The application of NiNPs as promising alternatives or combination to chlorhexidine, contribute to eradicate bacterial pathogens such as *S. mutans* in dental plaques at lower non-toxic levels. It is also suggested that more research be performed in this regard and with a larger sample size.

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Conflict of interest

None

List of abbreviations

NPs: nanoparticles
NiNPs: nickel nanoparticles
S. mutans: Streptococcus mutans
MIC: minimum inhibitory concentration
MBC: minimum bactericidal concentration

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