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Evaluation of the Antibacterial Effects of Nickel Nanoparticles on Biofilm Production by *Streptococcus mutans*

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ABSTRACT

***Background*:** Dental caries is a biofilm-dependent disease mainly causes by cariogenic bacteria that colonize dental surfaces, especially *Streptococcus mutans*. Nickel is a safe metal element which routinely used in dental compounds. The antibacterial and anti-biofilm effects of nickel nanoparticles (Ni-NPs) have been determined in limited studies, but the anti-biofilm effects of Ni-Nps on *S. mutans* have not been investigated before, so this study was aimed to investigate the anti-biofilm effects of Ni-NPs on *S. mutans* ATCC 35668.

***Methods*:** Biofilm formation by *S. mutans* ATCC 35668 was assayed by Microtiter Dish Biofilm Formation Assay and absorbance was measured by ELISA reader at 550 nm. The amounts of biofilm formation were also measured in the presence of 1, 01 and 0.01mg/mL concentrations of Ni- NPs by the same protocol and the mean amounts were compared between groups. Eight replicates were considered for each experiment. Data was statistically analyzed by SPSS16 software.

***Results*:** According to the statistical analysis, the amounts of biofilm formation were significantly reduced in the presence of all the tested concentrations of Ni-NPs.

***Conclusion*:** The current findings showed the potent anti-biofilm effects of Ni-NPs even in a concentration as low as 0.01 mg/ mL, so it is proposed for different applications in dentistry, considering its anti-biofilm effects. However, further studies on other biofilm producing members of oral microbiota including Lactobacilli are recommended.

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Introduction

Streptococcus mutans is a gram positive non-motile coccus and one of the first colonizers of the human oral cavity. Production of glucans, acid resistance, being successful in natural competence as well as the ability of this bacterium to form biofilm, makes *S. mutans* more successful in compare with the other oral primary colonizers. Because of having several virulence factors, *S. mutans* is the main etiological agent of dental caries (1). Dental caries is considering as one of the most expensive-to treat biofilm-dependent diseases worldwide, which threatens the health and normal life of children as well as adults (2).

Biofilms are multi-cellular, surface-attached, highly dynamic communities of microbial cells with particular physiologic characteristics, which are firmly attach to a surface. The extracellular polymeric substances such as exopolysaccharides, proteins and nucleic acids produced by biofilm bacteria, make them resistant to different classes of antibiotics as well as several mechanisms of host defenses (3-5).

Dental caries is a biofilm-dependent disease mainly causes by cariogenic bacteria that colonize dental surfaces, especially *S. mutans*. Frequent consumption of carbohydrates especially sucrose stimulates the synthesis of extracellular polysaccharides which in turn results biofilm formation (6). Removing of biofilm bacteria from the dental surfaces remain a challenge (7). On the other hand antibiotics such as other chemical compounds cannot penetrate well to the biofilm of bacteria and because of emerging the multi-antibiotic-resistant bacteria; there is an increased interest in the designing of alternatives to antibiotics. One promising field in this respect is nanotechnology (8).

Nanotechnology is a new field for production of new types of materials with an interesting range of size with medical applications for example as antibacterial agents (9). Different metallic nanoparticles are considered as antibacterial as well as anti-biofilm agents such as copper, silver, and zinc nanoparticles (10-15), however, some of these metals are so expensive. Nickel is cheap enough for intensive use and the antibacterial activities of nickel nanoparticles (Ni-NPs) have been shown in some studies (16). Nano-Nickel such as other nanomaterials has a high surface-to-volume ratio which increases its interaction with microorganisms and improves its activity (17).

On the other hand nickel is routinely used in dental compounds to increase the rate of good quality root-fillings (18). Also there are several studies which show that using of nickel in dental materials is safe and the rate of clinically relevant adverse reactions including contact allergies to it as one of the dental metals are significantly low (19), So this study was aimed to investigate the anti-biofilm effects of Ni-NPs on *S. mutans* ATCC 35668.

Materials and methods

Bacterial isolate and Ni-NPs

S. mutans ATCC 35668 was bought from the bacterial culture collection of Scientific and Industrial Research Organization of Iran. Ni-NPs with the size of less than 100 nm, in the form of nano-powder, were purchased from Sigma-Aldrich, and after suspending in Tripticase Soy Broth (TSB, Merck) with defined concentration, were ultrasonicated for two hours.

Microtiter Dish Biofilm Formation Assay

Biofilm formation assay was performed by the method illustrated by O'Toole (20), by culturing the microorganism in TSB for 24 h at 37°C in U-bottomed shape 96-well dishes. Crystal violet solution in water (0.1%) was used to stain the biofilm and acetic acid solution (30% in water) was used as solving agent and blank. Absorbance was measured by ELISA reader (Awareness Technology Inc) at 550 nm. Negative control was set by measuring the absorbance of TSB medium stained by crystal violet without adding any bacteria to the well. Eight replicates were considered for the experiment. Interpretation of

biofilm production was based on the criteria described elsewhere (21).

Susceptibility of biofilm to Ni-NPs

1, 01 and 0.01mg/mL concentrations of Ni- NPs were added to separate wells for the same isolate. Microtiter Dish Biofilm Formation Assay was performed in the presence of Ni- NPs by the same protocol by preparing eight replicates (20, 22).

Statistical analysis

The mean optical density obtained for the *S. mutans* ATCC 35668 in the presence of 1, 01 and 0.01mg/mL concentrations of Ni- NPs or absence of Ni- NPs. The data was statistically analyzed by statistical package for social sciences (SPSS16) software. One-way analysis of variance (ANOVA) was used for analyzing the differences between groups means. Differences between means considered statistically significant if the p-value was less than 0.05.

Results

Effects of Ni-NPs on S. mutans ATCC 35668 biofilm growth

Figure 1 shows the mean optical density± SD for each group in the presence of 1, 01 and 0.01mg/mL concentrations of Ni- NPs. According to the statistical analysis, the effects of 1, 0.1, and 0.01 mg/ mL of Ni-NPs on *S. mutans* biofilm reduction were significant (P=0.000, 0.004 and 0.009 respectively), so all the concentrations of Ni-NPs reduced biofilm formation significantly.

Discussion

Nickel-containing alloys are routinely used in dentistry in order to form the space maintainers, brackets, fillings, and crowns (23, 24). The harmful effects of Ni mostly relate to its allergenicity, and it was shown that almost about 10% of the general population show an allergic reaction to this metallic element with a more frequency in females than males (25). The toxic amount of nickel is 50 - 500 mg/kg of body weight and the daily uptake of this element is approximately 200–300 µg (26).

S. mutans is well defined as the main microorganism in development of dental plaques in human, which may result in subsequent caries formation (22). The adherence of *S. mutans* to the tooth surfaces is performed by biofilm production, which is formed on the basis of the conversion of dietary sucrose to insoluble glucans mediated by bacterial enzymes (27). The ability of biofilm formation, acidogenicity and acid tolerance are the main virulence factors of this microorganism (1, 28).

Some metal nanoparticles are active against microorganisms (10-13, 29-32); either by killing, inhibiting of their growth or eradication of bacterial biofilms (33-35). Silver NPs are potent killers of biofilm bacteria (22, 36), but they are expensive enough for routine usage in dental practices.

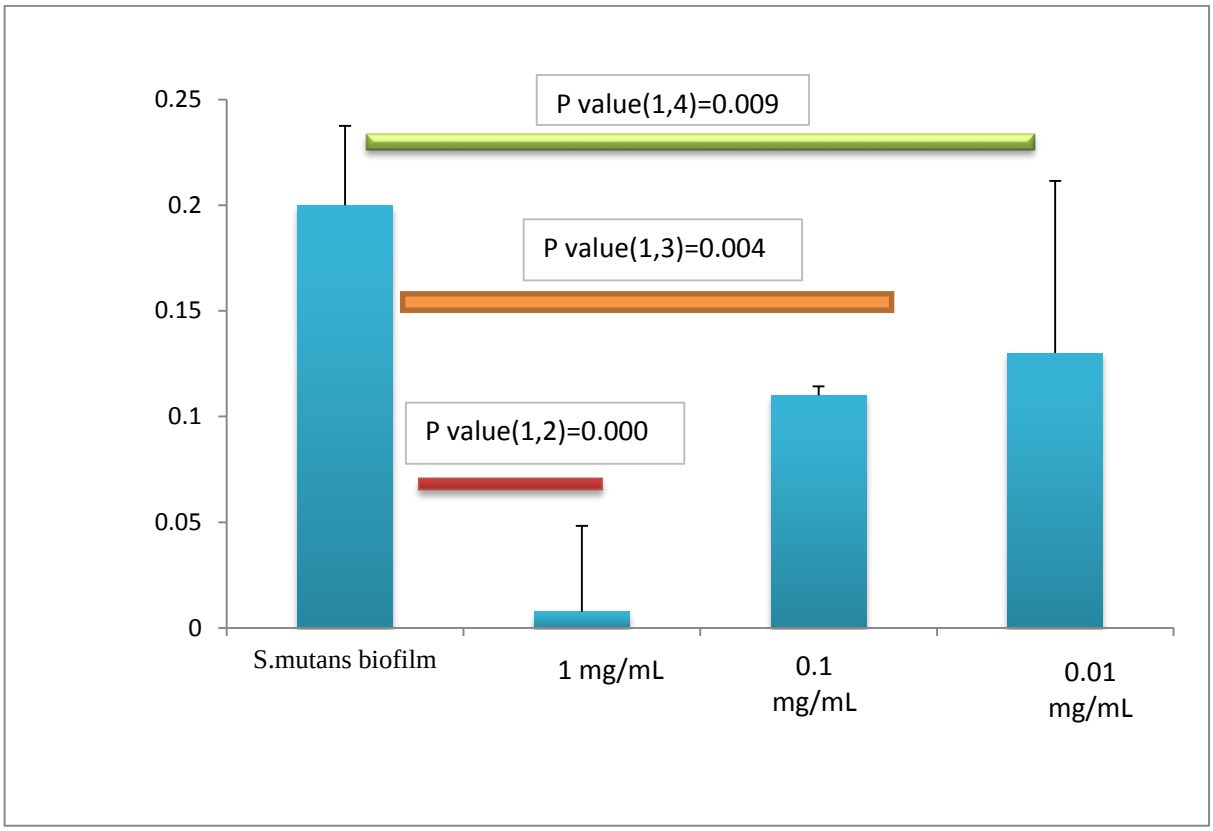

Figure 1. Biofilm formation by *S. mutans* ATCC 35668 in the presence/ absence of Ni-NPs.

Argueta-Figueroa et al. investigated the antibacterial effects of Ni-NPs and copper-nickel nanoalloys on some bacterial strains of oral microbiota including *S. aureus* and *S. mutans*. They demonstrated the similar antibacterial effects of both compounds and concluded that NPs are partly active against tested strains and proposed usage of these NPs in dentistry (37), however, up to our knowledge the anti-biofilm ability of Ni-NPs on caries bacteria including *S. mutans* has not been investigated so far. The current findings showed the potent anti-biofilm effects of Ni-NPs even in a concentration as low as 0.01mg/ mL, in a way that the biofilm formation was significantly decreased, it was mentioned before that in order to create any mucosal allergic reactions, the allergen should be 5–12 times more concentrated than the concentration which is needed to produce an allergic reaction on the skin (24), so using of Ni -NPs in the studied concentration is anti-biofilm effective and safe enough for different applications in dentistry.

Conclusion

The current findings showed the potent anti-biofilm effects of low concentrations of Ni-NPs, so it is recommended for different applications in dentistry, considering its anti-biofilm effects. However, further studies on other biofilm producing members of oral microbiota including Lactobacilli are recommended.

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Ethical Considerations

The study was approved by Ethics Committee of Urmia University of Medical Sciences, Urmia, Iran.

Conflict of interest

The authors declare no conflicts of interest.

Financial disclosure

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