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Original research

Evaluation of the antibacterial effect of nickel oxide nanoparticles against bacteria involved in dental caries

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Abstract

Tooth decay is one of the most common diseases in the oral cavity and is one of the most widespread diseases in the human population. This study aimed to determine the antibacterial effect of nickel oxide nanoparticles against bacteria involved in tooth decay. In this study, the disk diffusion method was used to determine the antibiotic susceptibility and the microdilution broth method was used to determine the minimum inhibitory concentration (MIC). Nanoparticles were also synthesized in two molecular size (A: 8.1 and B: 12 nm) by the sol-gel method. The MIC of the first nanoparticle for *Streptococcus sanguinis* and *Streptococcus mutans* was 31.25 and 125 µg/ml, respectively. The MIC of the second nanoparticle for *S. sanguinis* was 125 µg/ml. In the case of *S. mutans* up to a concentration of 500 µg/ml, no growth inhibition was observed. The results showed that nickel oxide nanoparticles have acceptable antibacterial properties against *S. mutans* and *S. sanguinis*, which can be used in dental materials to prevent dental caries. However, this requires the determination of cellular toxicity and its side effects in future studies.

Keywords: Nanoparticles, Nickel oxide, *Streptococcus sanguinis*, *Streptococcus mutans*

1. Introduction

Tooth decay is one of the most common diseases in the oral cavity and is one of the most widespread diseases in the human population. About half of people 6 to 19 years old who are economically disadvantaged have decayed teeth [1]. Tooth decay is also a problem for adults, with more than 90% of people over the age of 40 having it. A quarter of people over the age of 60 have lost all their teeth permanently due to the impact of caries on their self-esteem and involvement in nutritional problems [2]. Bacteria are the main cause of tooth decay. The main mechanism of bacteria in causing tooth decay is the formation of plaque on tooth enamel. Plaque is actually a kind of biofilm that is made up of one or more types of microorganisms that can grow at different levels. The formation of dental biofilm is not necessarily a sign of

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disease, but the formation of biofilm is an important mechanism of disease for bacteria [3].

Streptococcus mutans is a Gram-positive and aerobic cocci in the oral cavity flora in humans. This bacterium is the most important cause of tooth decay. *S. mutans* damages tooth enamel by fermenting sucrose and producing lactic acid. The bacterium also uses sucrose to make dental plaque. *S. mutans* is known to produce lactic acid as part of its metabolism [4, 5]. Unlike most edible microorganisms, *S. mutans* grows in low acid conditions and becomes the dominant bacterium in cultures with a permanent decrease in pH. In addition, contrary to the characteristics of plaque, whose metabolism is significantly slowed down at such a pH, the metabolism of *S. mutans* is actually improved [4, 6]. *Streptococcus sanguinis* is a Gram-positive, aerobic cocci and normal oral flora and is found in dental plaque. *S. sanguinis* is widely present in the oral cavity [7-9]. Studies show that *S. sanguinis* has two genes which produces glucan. Glucan is also formed in the presence of glucose and various forms of sucrose. Glucan is the major biofilm polymer in *S. sanguinis* and increases the adhesion of bacteria to hydroxyapatite, promoting biofilm development [10, 11].

When particles are prepared in nanoscale (less than 100 nm) they can react with organic and inorganic molecules more easily due to the increase in surface area [12]. The main advantages of hydroxyapatite (HA) nanoparticles are their similarity to tooth mineral structure, biological activity and biocompatibility [13, 14]. In one design, the effect of hydroxyapatite magnesium carbonate nanopowder was used to prevent and repair primary caries lesions and the results showed that MgHa nanoparticles are adsorbed on the enamel surface and by changing the mechanical properties of the tooth, a new layer is formed in Enamel surface and filling all cavities improves tooth remineralization [15]. Nickel oxide is of interest due to the chemical and magnetic properties of an oxide. Nickel oxide nanoparticles are widely used. These nanoparticles are also used as an effective catalyst for the oxidation of many organic compounds. Nickel oxide nanoparticles as an antibacterial agent have also been effective on some bacteria. Numerous methods for the preparation of nickel oxide nanoparticles are known. The size distribution and morphology of nanoparticles depend on the way they are synthesized. Among the nanoparticle synthesis methods, we can mention the most common methods called sol-gel method [16].

Removing plaque from teeth reduces the risk of problems such as tooth decay, gum disease and bad breath. Since aerobic and anaerobic bacteria are an important part of dental plaque, new substances with antibacterial properties are essential to prevent their growth. Metal nanoparticles show significant antibacterial activity. This property is due to the very small size and surface to volume ratio of these particles. In this regard and the lack of similar studies, we aimed to investigate the antibacterial effect of nickel oxide nanoparticles against bacteria involved in tooth decay.

2. Materials and Methods

2.1 Bacterial strains

The studied strains were standard ATCC of *S. mutans*, and *S. sanguinis* which was lyophilized from the Center for Genetic and Biological Resources of Iran. To prepare the strain for the experiment, the stored strain was first inoculated in 5 ml of tryptone soy broth (Merck, Germany) and incubated for 24 hours at 37 °C. Then, a fresh culture of a bacterial suspension equal to half McFarland standard was prepared in Müller-Hinton broth medium and used for further experiments.

2.2 Synthesis of nickel oxide nanoparticles

At first, 100 ml of 2 M ammonium hydroxide solution dropwise to 50 ml of 0.5 M solution of Ni (NO3) 2.6H2O stirred by magnetic magnet for 4 h at 100 °C. C was added. The solution was kept at room temperature for 24 hours. The prepared light green suspension was centrifuged and then the dry precipitate was calcined at 300 and 700 °C (17) [17]. The structure and morphology of the synthesized nanoparticles were identified by FTIR and TEM techniques. The size of synthetic nanoparticles was estimated to be about 5 to 15 nanometers with this technique. The synthesized nanoparticle powder was first weighed by a sensitive scale on sterile aluminum foil. Then pour one gram of nanoparticles in a sterile tube 16 x 20 mm screw cap, and add 10 ml of sterile distilled water. After vortexing, we mixed it well in the sonicator and at room temperature for 10 minutes. Then the desired serial dilutions were prepared. All

steps were performed in full compliance with sterile conditions.

2.3 Determination of antibiotic susceptibility

In order to determine the antibacterial susceptibility, well diffusion method on Muller-Hinton agar (MHA; Merck, Germany) described by Nanda et al. were applied. In each well 25 µl of nanoparticles in desired concentrations (500, 250, 125, and 62.5 µg/ml) was added [18]. Finally, the plates were transferred to an incubator at 35-37 °C and the results were evaluated after 16-18 hours. Finally, the results were reported based on the measurement of the diameter of the growth inhibition zone by a millimeter ruler.

Determination of minimum inhibitory concentration (MIC) was performed by standard broth microdilution based on the clinical and laboratory standards institute (CLSI) recommendation [19]. First, 96-well microplates different concentrations of nanoparticles (500-15.625 µg/mL) were provided using Müller-Hinton broth (Merck, Germeny) medium containing 5% Sheep blood agar. After providing the required concentrations, 10 µl of the diluted microbial suspension was added to each well (according to CLSI method), the optimum concentration of bacteria for inoculation into the wells is 10^6 CFU/ ml. Following 24 h incubation at 37 °C, the wells were inspected for microbial growth, and the MIC results were recorded based on the lowest concentration that did not produce visual growth.

2.4 Statistical analysis

Analysis was performed by using SPSS™ software, version 22.0 (IBM Corp., USA). The results are presented as descriptive statistics in terms of relative frequency.

3. Results

Morphological studies determined the size of calcifying nanoparticles at 300 °C (NP1) and 400 °C (NP2) were 8.1 and 12 nm, respectively. As the calcination temperature decreases, the size of the nanoparticles decreases, and as the size decreases, the surface-to-volume ratio increases.

According to the Table 1, the growth inhibition zone for the concentration of 500 to 62.5 µg/ml of nanoparticles for *S. sanguinis* varied from 12 mm to 17 mm. For *S. mutans*, the minimum zone diameter was 10 mm at the 250 µg/ml of nanoparticles and the maximum zone diameter was 14 mm at both 125 and 62.5 µg/ml of nanoparticles. Based on Table 2, the amount of the first MIC nanoparticle for *S. sanguinis* and *S. mutans* was 31.25 and 125 µg/ml, respectively.

4. Discussion

According to our results, it was illustrated that *S. sanguinis* represented a higher antibacterial effects than *S. mutans*. Numerous studies have been performed on the effects of nanocomposites on bacteria that cause tooth decay. Similar to our study, a study by Khodaee et al., the antibacterial and anti-biofilm effects of silver nanoparticles against dental plaque microorganisms, *S. mutans* and *Agrigatebacter Actinomycetemcomitans* represented that the active ingredient in silver nanoparticles showed a greater antibacterial effect than nickel oxide nanoparticles [20]. In the study of Emrani et al., the results showed that the MIC for biosynthesized nanoparticles of licorice extract against *S. mutans*, *Actinomyces viscosus* and *Lactobacillus rhamnosus* bacteria were 1.56, 6.25, and 50 µg/ml, respectively ($P \leq 0.05$), and the MIC for nanoparticles biosynthesized with peppermint extract was 12.5, 12.5, and 200 µg/ml ($P \leq 0.05$), respectively [21]. Both expression and mint showed greater antibacterial effect than nickel oxide nanoparticles.

In another study conducted by Vahid Dastjerdi et al., it was found that the largest diameter of the growth inhibition disk in the disk diffusion method for *S. mutans*, *S. sanguinis*, *Streptococcus salivarius*, *Streptococcus sobrinus* and *Enterococcus faecalis* at a concentration of 100 micrograms per milliliter and equal to 14, 18, 15, 18, and 10.5 µg/ml, respectively. The MIC values for *S. mutans*, *S. sanguinis*, *S. salivarius*, *S. sobrinus* and *E. faecalis* were 50, 25, 6.25, 25, 25, and 50 µg/ml, respectively [22]. Similar to the previous study on nanoparticles biosynthesized with licorice and peppermint, in this study the results showed that the aqueous extract of pomegranate flower showed slightly more antibacterial properties than nickel oxide nanoparticles, and perhaps this hypothesis suggests that the use of biological compounds along with nanoparticles increases their effectiveness, which of course requires further studies to prove this.

Table 1. Diameter of zone inhibition by well-diffusion

Nanoparticle	*Streptococcus sanguinis*				*Streptococcus mutans*			
	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml
NP (1)	12mm	12	16	17	11mm	10	14	14
NP (2)	11	12	14	14	0	0	12	12

Table 2. The Minimum inhibitory concentration (MIC) of nanoparticles

Nanoparticle	MIC	
	Streptococcus sanguinis	*Streptococcus mutans*
NP (1)	31.25 µg/ml	125 µg/ml
NP (2)	125 µg/ml	>500 µg/ml

In another study conducted by Sadeghi et al., the MIC of silver and chlorhexidine nanoparticles in relation to *S. sanguinis* was 25 and 16 µg/ml, respectively, and for *Actinomyces viscosus* was 4 and 64 µg/ml, respectively. Comparing the results of this study with the present study, both types of nickel oxide nanoparticles showed more antibacterial effect than chlorhexidine and less antibacterial effect than silver nanoparticles [23].

An investigation by Lu et al., in which the MIC of 5 nanometer silver nanoparticles for anaerobic bacteria such as *Actinomycetemcomitans*, *Fusobacterium nucleotum*, *Streptococcus mitis*, *S. mutans*, and *S. sanguinis* were 50, 50, 25, 25, 25 and 25 µg/ml, respectively [24]. Comparing the results of this study with our study, showed that the first type of nickel oxide nanoparticles showed less antibacterial effect against *S. mutans* and more antibacterial effect against *S. sanguinis* than silver nanoparticles. In the study of Aflatoonian et al., the minimum growth inhibitory concentration and the minimum bactericidal concentration of nanoparticles on *Pseudomonas aeruginosa* and *E. faecalis* strains were determined using broth microdilution method. Visible-ultraviolet spectroscopy showed an absorption peak in the range of 370 nm. Transmission electron microscopy images showed the synthesis of more spherical zinc oxide nanoparticles with a size of less than 50 nm. The lowest inhibitory concentrations of ZnO nanoparticles against *P. aeruginosa* and *E. faecalis* were determined to be 6.25 and 12.5 µg/ml, respectively [25]. A study by Davari et al. represented that copper and zinc oxide nanoparticles at concentrations of 0.1 and 0.5% at 15 and 30 days compared to the control group significantly reduced the number of bacteria. Zinc oxide nanoparticles at 0.5% in the composite composition showed the highest and both oxidized nanoparticles and zinc at the concentration of 0.05% showed the lowest amount of antimicrobial properties [26].

Despite the promising effects of nickel oxide nanoparticles, further study to investigate the toxicity on different human cell lines is suggested as the most important limitation of the recent study.

The results showed that nickel oxide nanoparticles have acceptable antibacterial properties against *S. mutans* and *S. sanguinis*, which can be considered as preventative agents for tooth decay in materials and compounds used in dentistry.

Authors' contributions

Concept and Study design: EK, MH, FN, HP; Methods, data collection and experimental work: EK, MJ, MH; Results analysis and drafting: CT, SN, HG, HR, HP; Critical revisions: CT, MH, HR, FN, HP. All authors read and approved the final version.

Conflict of interests

None to be declared.

Ethical declarations

The study deign was approved by the ethical committee at the Guilan University of Medical Science [IR.GUMS.REC.1399.569].

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References

1. Allaker RP, Memarzadeh K. Nanoparticles and the control of oral infections. Int J Antimicrob Agents. 2014;43(2):95-104.
2. Song W, Ge S. Application of antimicrobial nanoparticles in dentistry. Molecules. 2019;24(6):1033.
3. Costerton JW, Cheng KJ, Geesey GG, Ladd TI, Nickel JC, Dasgupta M, et al. Bacterial biofilms in nature and disease. Annu Rev Microbiol. 1987;41(1):435-64.
4. Lemos JA, Palmer SR, Zeng L, Wen ZT, Kajfasz JK, Freires IA, et al. The Biology of Streptococcus mutans. Microbiol Spectr. 2019;7(1):1-18.
5. Napimoga MH, Kamiya RU, Rosa RT, Rosa EA, Höfling JF, de Oliveira Mattos-Graner R, et al. Genotypic diversity and virulence traits of Streptococcus mutans in caries-free and caries-active individuals. J Med Microbiol. 2004;53(Pt 7):697-703.
6. Krzyściak W, Jurczak A, Kościelniak D, Bystrowska B, Skalniak A. The virulence of Streptococcus mutans and the ability to form biofilms. Eur J Clin Microbiol Infect Dis. 2014;33(4):499-515.
7. Caufield PW, Dasanayake AP, Li Y, Pan Y, Hsu J, Hardin JM. Natural history of Streptococcus sanguinis in the oral cavity of infants: evidence for a discrete window of infectivity. Infect Immun. 2000;68(7):4018-23.
8. Gross EL, Beall CJ, Kutsch SR, Firestone ND, Leys EJ, Griffen AL. Beyond Streptococcus mutans: dental caries onset linked to multiple species by 16S rRNA community analysis. PLoS One. 2012;7(10):e47722.
9. Seoudi N, Bergmeier LA, Drobniewski F, Paster B, Fortune F. The oral mucosal and salivary microbial community of Behçet's syndrome and recurrent aphthous stomatitis. J Oral Microbiol. 2015;7:27150.
10. Yoshida Y, Konno H, Nagano K, Abiko Y, Nakamura Y, Tanaka Y, et al. The influence of a glucosyltransferase, encoded by gtfP, on biofilm formation by Streptococcus sanguinis in a dual-species model. APMIS. 2014;122(10):951-60.
11. Zhu B, Ge X, Stone V, Kong X, El-Rami F, Liu Y, et al. ciaR impacts biofilm formation by regulating an arginine biosynthesis pathway in Streptococcus sanguinis SK36. Sci Rep. 2017;7(1):17183.
12. Jeevanandam J, Barhoum A, Chan YS, Dufresne A, Danquah MK. Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. Beilstein J Nanotechnol. 2018;9:1050-74.
13. Amiri M, Etemadifar Z, Daneshkazemi A, Nateghi M. Antimicrobial Effect of Copper Oxide Nanoparticles on Some Oral Bacteria and Candida Species. J Dent Biomater. 2017;4(1):347-52.
14. Nozari A, Ajami S, Rafiei A, Niazi E. Impact of Nano Hydroxyapatite, Nano Silver Fluoride and Sodium Fluoride Varnish on Primary Teeth Enamel Remineralization: An In Vitro Study. J Clin Diagn Res. 2017;11(9):ZC97-ZC100.
15. Noori AJ, Kareem FA. The effect of magnesium oxide nanoparticles on the antibacterial and antibiofilm properties of glass-ionomer cement. Heliyon. 2019;5(10):e02568.
16. Imran Din M, Rani A. Recent Advances in the Synthesis and Stabilization of Nickel and Nickel Oxide Nanoparticles: A Green Adeptness. Int J Anal Chem. 2016;2016:3512145.
17. Zorkipli NN, Kaus NH, Mohamad AA. Synthesis of NiO Nanoparticles through Sol-gel Method. Procedia Chem. 2016;19:626-31.
18. Nanda A, Saravanan M. Biosynthesis of silver nanoparticles from Staphylococcus aureus and its antimicrobial activity against MRSA and MRSE. Nanomedicine. 2009;5(4):452-6.
19. Wayne PA. Performance Standards for Antimicrobial Susceptibility Testing. Clinical and Laboratory Standards Institute (CLSI) 2022; 32th Informational Supplement. (M100-S28).
20. Khodaee S, Sadeghi R, Taleghani F, Mahboubi A, Shafaei Mobarakeh A. In Vitro Assessment of Anti-Bacterial and Anti-Biofilm Effects of Nanosilver Solution on Dental Plaque Microorganisms. J Mashhad Dent Sch. 2020;44(2):114-26.
21. Emrani S, Zhiani R, Dafe Jafari M. The biosynthesis of silver nanoparticles using plants of Glycyrrhiza glabra and Mentha piperata and its antimicrobial effect on some bacterias that cause tooth decay. J Rafsanjan Univ Med Sci. 2018;16(10):953-68.
22. Vahid Dastjerdi E, Abdolazimi Z, Ghazanfarian M, Amdjadi P, Kamalinejad M, Mahboubi A. Effect of Punica granatum L. Flower Water Extract on Five Common Oral Bacteria and Bacterial Biofilm Formation on Orthodontic Wire. Iran J Public Health. 2014;43(12):1688-94.
23. Sadeghi R, Owlia P, Taleghani F, Sharif F. An in-vitro comparison between antimicrobial activity of nanosilver and chlorhexidine against Streptococus sanguinis and Actinomyces viscosus. J Iran Dent Assoc. 2011;23(4):225-31.
24. Lu Z, Rong K, Li J, Yang H, Chen R. Size-dependent antibacterial activities of silver nanoparticles against oral anaerobic pathogenic bacteria. J Mater Sci Mater Med. 2013;24(6):1465-71.
25. Aflatoonian M, Khatami M, Sharifi I, Pourseyedi S, Yaghobi H, Naderifar M. Evaluation antimicrobial activity of biogenic zinc oxide nanoparticles on two standard gram positive and gram negative strains. Tehran Univ Med J. 2017;75(8):562-9.
26. Davari A, Mosaddegh A, Daneshkazemi A, Mortazavi Sanigei SM. Comparison of Antibacterial Effect of Composite Resins Incorporating Copper with Zinc Oxide Nanoparticles on Streptococcus Mutans. J Mashhad Dent Sch. 2020;43(4):344-51.