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Incorporation of zinc oxide nanoparticles and its antibacterial effect on toothpaste

Amal M. El Shahawi^{1*}

Abstract

Background Dental caries is the most prevalent oral infection affecting the individuals worldwide, and *Streptococcus mutans* is the major microorganism involved in its pathology. Thus, the aim was to evaluate the antibacterial effect of addition of nanozinc particles on toothpaste with different concentrations. The study was carried out as Deburden toothpaste was used as a control group, and nanozinc particles were added with different concentrations to the same toothpaste, and antibacterial test for each group was evaluated.

Methods Group 1: (control group) toothpaste only. Group 2: 0.5% of nanozinc particles added to toothpaste. Group 3: 1% of nanozinc particles added to toothpaste. The three groups were incubated for 24 h at 37 °C, and the antibacterial test was tested for all groups using agar well diffusion method.

Results All the samples had antibacterial effect against *Streptococcus mutans*. Meanwhile, Group 3 has showed the greatest zone of inhibition compared to the control group showed the lowest effect.

Conclusions One % of nanozinc particles were more efficient on *Streptococcus mutans* in comparison with 0.5% nanozinc particles concentration effect.

Keywords Nanoparticles, Toothpaste, *Streptococcus mutans*, Antibacterial test

Background

One of the main common prevalent diseases over all the world is dental caries (Anusavice 2002; Yoo et al. 2007) which result from reaction of certain bacteria with ingredients of the diet within a biofilm designated “dental plaque” (Bowen 2002). The main etiologic agent of dental caries is bacterial plaque on dental surfaces and contain oral flora. And despite the great work in the oral health community, dental caries still remains common cause of the present dental diseases (Van Gemert et al. 2008).

Streptococcus mutans is a type of bacteria that is considered the main cause of dental caries through

destruction of the outer dental structures as a result of acids production (Loesche 1986). One of the risk factors in the induction of dental disorders is colonization of bacteria on teeth (Loesche 1986). *Staphylococcus* (*S. aureus* and *S. epidermidis*) as a major human pathogen, which is one of the main causes for a number of infections which exists mainly in the mouth and extremities which are main sources for proliferation of this pathogen (Knighton 1986; Lowy 1998; Piochi and Zelante 1974; Rodis et al. 2006). *Streptococcus mutans* bacteria which is severely colonized in some individuals are considered to be with high caries index. Hence, extermination of these microorganisms is important for dental treatment (Rodis et al. 2006). Reaction of bacteria occurs by various methods including accumulation (Kolenbrander et al 2000), cell–cell communication and metabolic exchange (Li et al. 2001a; Roberts et al. 2001). These mechanisms helps in persistence of bacteria and their colonization, and also destruction of enamel, dentin or cementum of

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teeth occurs due to bacterial activity which caused by dental caries (Sutherland 2001; Paster et al. 2001; Li et al. 2001b).

Dental caries is a dependent oral disease in which fermentable dietary carbohydrates such as sucrose are the vital environmental factors involved in its induction and propagation which also aids as a substrate for the synthesis of polysaccharides in dental plaque (Yoo et al. 2007; Newbrun 1967).

Nanotechnology is an advanced field to diagnose and cure different diseases (Rezaei et al. 2019) to enhance curing, diagnosis and treatment of oral and dental diseases (Das and Nasim 2017; Sharan et al. 2017), and a lot of dental materials have been used in the nanoscale to improve their properties in different forms such as nanofibers, nanopores, nanorods, nanoparticles, nanorobotic dentifrice, nanosolutions, and nanoneedles.

Zinc oxide (ZnO), titanium dioxide (TiO₂), and silver are metal NPs which are categorized by their size, composition, crystallinity and structure. Their dimensions are modified into nanoscale which can alter their chemical, mechanical, electrical, structural, morphological, and optical properties. These modified structures allow the nanosized particles with high concentration to interact with the internal atoms and consequently enable the transmission of NPs into the inner cellular structures physically as they have higher surface activity (Rasmussen et al. 2010).

Studies have shown that zinc oxide can inhibit acid production by *Streptococcus mutans* and *Lactobacillus* in dental plaque (Hirota et al. 2010). Also, it was reported that it has antibacterial effects on Gram-negative and Gram-positive bacteria and is commonly used as an antibacterial agent in dental hygiene products such as toothpastes and mouthwashes due to increased surface/volume ratio.

Nanoparticles can act as an efficient antibacterial agent and is widely accepted in biomedicine. The superior bactericidal activity of NPs with antibacterial activities is attributed to their electrostatic attraction between positively charged NPs and has the potential to reduce or eliminate the evolution of more resistant bacteria. The small size of NPs improves not only their antimicrobial action with minimal adverse effects, including hypersensitivity and allergic reactions, but also their mechanical properties. Many studies investigated the antibacterial effect of NPs combined with a wide range of dental materials (Ferrando-Magraner et al. 2022).

Zinc oxide (ZnO) was chosen on this study as it is a mineral zincite that is bio-safe, biocompatible with

proven strong anti-bacterial properties, which can powerfully resist broad range of microorganisms due to their ability to generate reactive oxygen species (ROS) on the surface of oxides.

The aim of the study is to evaluate the effect of adding nanozinc oxide as an antibacterial agent to tooth paste.

Methods

Sample preparation

In the present study, 15 samples were prepared and divided into three main groups (five samples each)

Group One (control group) Toothpaste alone (Deburdent).

Group Two Toothpaste (Deburdent) with 0.5% nanozinc particles (0.1 wt%).

Group Three Toothpaste (Deburdent) with 1% nanozinc particles (0.2 wt%).

Zinc oxide nanoparticles production and description

ZnO nanoparticles were prepared by refluxing precursor zinc acetate dihydrate (0.1 M) in diethylene glycol and triethylene glycol at 180 °C and 220 °C, respectively. Reaction time varied for 2 and 3 h with and without sodium acetate (0.01 M). Before refluxing, the solution was kept on a magnetic stirrer at 80 °C for 1.5 h. After completion of reflux action, the samples were centrifuged at 8000 rpm for 15 min and washed with distilled water and ethanol for three times. Further, it was dried at 80 °C for overnight.

The nanozinc particles are added with concentrations 0.5 and 1%, respectively, to each sample and mixed well before the antibacterial testing.

Sample preparation

Zinc oxide nanoparticles were weighed corresponding to the ratio of 0.5% and 1% by using sensitive balance, dilution of toothpaste with distilled water by ratio 1:1; then, every sample consists of 2 g of toothpaste that was diluted with 2 ml of distilled water.

Bacteria preparation

Antibacterial test

Activation of bacterial strains The strain of *Streptococcus mutans* ATCC 25,175 (Microbiological Resources Centre, Cairo MIRCEN, Egypt) was used in this study. The *S. mutans* was activated in tryptone soya broth at 37 °C overnight.

Group 1 (plain toothpaste) is added to bacteria incubated in blood agar.

Group 2 (0.5% ZnO added to toothpaste) is added to the bacteria agar.

Group 3 (1% ZnO added to toothpaste) is added to the bacterial agar.

Antibacterial testing preparation

Antimicrobial activity of toothpaste using agar well diffusion method.

The activity of toothpaste (control group, 0.5% and 1%) was determined by well diffusion test according to Elgamily et al. (2018) using *Mitis salivarius*-bacitracin agar. *Mitis salivarius*-bacitracin agar was poured into sterile Petri dishes (20 ml each), and the tested *S. mutans* (around 20 µl of 10⁴ CFU) were sprayed by sterile swab regularly on the surface of agar medium. The wells with 6 mm were prepared in the medium. Each well was filled with 100 µl of each toothpaste. All plates were incubated for 24 h at 37 °C; after the incubation period, the antimicrobial activity was evaluated by measuring the diameters of zones of inhibition (in mm) (Elgamily et al. 2018) as shown in Fig. 1.

Data analysis

Statistical analysis was done to all groups using the analysis of variance (ANOVA) and the Tukey’s test using the statistical software SPSS, version 10.0

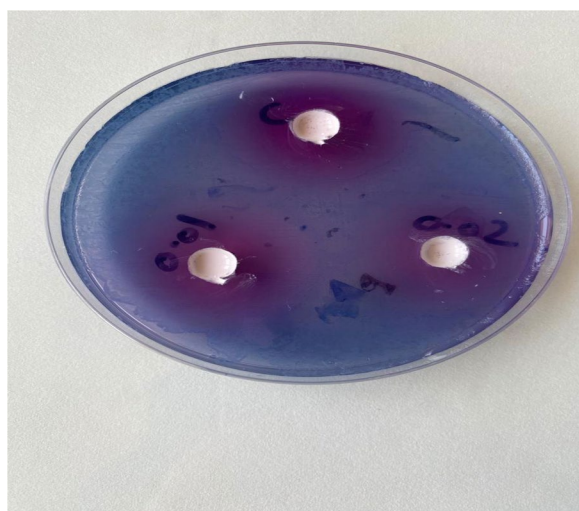


Fig. 1 Samples introduced into agar plates and kept for incubation



Fig. 2 Zones of inhibition after 24-h incubation

Results

After 24 h, the three incubation plates were observed as shown in Fig. 2

Group 1 plain toothpaste shows minimal antibacterial zone (16.7 mm).

Group 2 0.5% ZnO with toothpaste shows moderate antibacterial zone (21.1 mm).

Group 3 1% ZnO with toothpaste shows the maximum antibacterial zone (24.7 mm).

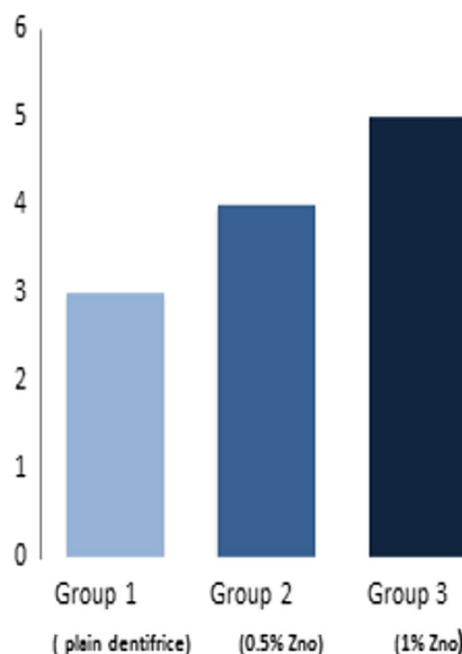


Fig. 3 Graph bar for the three groups

Therefore, group 3 showed the highest antibacterial effect followed by group 2 and group 1 (control group) showing the least antibacterial effect with minimal bacterial count (Fig. 3).

Discussion

Streptococcus mutans (*S. mutans*) has a main role in the occurrence of dental caries. The main cause of *S. mutans* pathogenesis is its capability to produce big amount of glucans and acid that is greater than the salivary cushioning abilities. *S. mutans* has a big ability to adhere to teeth and can persist in an oral condition which is acidic (Matsumoto-Nakano et al. 2018) so it was the cause of its choice in this study.

Toothpaste (2 gm) was diluted with 2 ml of distilled water as the effective antibacterial activity may not be better to establish to have a minimal inhibition zone diameter as toothpaste used in vivo is usually diluted by saliva, the level to which antimicrobial properties are buffered or lost in dilution in vitro is of interest (Inetianbor et al. 2014). In addition, various toothpaste at different rates can create different active ingredients and may diffuse so that, it should be considered that any toothpaste brand have an average inhibition zone which may not be directly with that of other toothpastes.

All the samples with toothpaste showed results against the tested dental bacterial pathogens with group 3 showed the highest inhibition zone on the average (24.7 mm) followed by group 2 (21.1 mm), while group 1 showed the minimal activity (16.7 mm). Numerous previous studies have confirmed the inhibitory effects of the antibacterial toothpaste (Deburdent) on oral bacteria (Fine et al. 2006).

Several studies have reported the efficiency of cleaning with antibacterial toothpaste and mouthwash in remarkable decrease in mucosal and salivary levels of bacteria. Accordingly, previous results have revealed that several types of toothpastes showed various antibacterial actions levels for both toothpastes having active and inactive compositions. This is likely due to alterations in formulations, the active ingredients concentration and its interaction with other constituents. However, these results identify the antibacterial effect of mouthwashes reported through previous studies (Shaei et al. 2018; Fine et al. 2006).

Deburdent toothpaste showed antimicrobial effect may be due to the presence of bioactive particles in its composition, although the presence of nanozinc particles added to the same toothpaste showed higher antibacterial effect.

Nanoparticles play an essential role as effective antimicrobial agent and is well-known in the dental and oral medicine field. The high antibacterial and also bactericidal activity is due to its positively charged NPs which

are attracted to each other electrostatically and have the capability to decrease the formation of more antibacterial agents, as their mechanism of action is always in contact with the bacterial cell wall and at the same time penetrates certain internal structures (Ferrando-Magraner et al. 2022). This high antimicrobial capacity could be related to their size and high surface area. However, the small size of the particles is not the goal, and the use of different procedures of synthesis to obtain new physico-chemical properties are also related to variables affecting antibacterial activity so that nanozinc particles added with concentrations 0.5% and 1% were enough to fulfill the antibacterial effect on toothpaste used as shown in the results.

Zinc oxide in the form of nanoparticles shows an effective antibacterial properties. Due to increased surface/volume ratio of such nanoparticles, it shows higher antimicrobial action (Borzabadi-Farahani et al. 2014; Shaei et al. 2018). Several studies revealed that increasing the concentration of ZnO nanoparticles up to 1% showed the best antibacterial effect as zinc oxide aid in acid production inhibition caused by *Streptococcus mutans*. Furthermore, it has been known that some dental products are used nowadays as antibacterial agents with antibacterial effect such as dentifrices and mouthwashes. The target of this study was to evaluate the effect of zinc oxide addition among all the current metal oxide nanoparticles; zinc oxide (ZnO) is a mineral zincite that is biologically compatible with safety use, and they are capable to create reactive oxygen species (ROS) on the oxide surface, so that it was the main reason of selecting this type of nanoparticles.

Although zinc has an appropriate antibacterial effect, toxicological effect and possibly harmful effects on the human body may be one of the limiting factors of use of such nanoparticles in high concentration due to the various antibacterial concentration efficiency to *S. mutans*, so that it was safe up to 1–2% only; so that we tried two different concentrations only 0.5% and 1%. It may be not enough to use minimal amount of zinc oxide against *mutans* and also a higher concentration could produce cytotoxicity and genotoxicity. Dental care products containing ZnO particles present a major source of contact with tooth surface as these products are used by patients on regular basis daily. Therefore, wide consideration is to be in mind toward whether zinc oxide in the form of nanoparticles in these forms are capable to interact with the barrier of oral mucosa and causes inflammation and systemic toxicity (Safaei et al. 2020; Sirelkhatim et al. 2015).

Conclusions

Within the limitation of this study, it is shown that the addition of nanozinc particles to toothpaste demonstrated marked antibacterial activity against the test

organism (*Streptococcus mutans*) with different concentrations. 1% of nanozinc particles showed the highest antibacterial effect on toothpaste.

Recommendation

Modifications with different concentrations of nanozinc particles can be recommended up to 2% for further investigations on antibacterial effect and different side effects on different toothpastes.

Abbreviations

ZnO Zinc oxide
NPs Nanoparticles

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Author contributions

All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The author declare that they have no competing interests.

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References

- Anusavice KJ (2002) In-vitro antibacterial activity of commonly used toothpastes in Nigeria against dental pathogens. *Edu Dent* 23:12–20
- Borzabadi-Farahani A, Borzabadi E, Lynch E (2014) Nanoparticles in orthodontics, a review of antimicrobial and anti-caries applications. *Acta Odontologica Scandinavica* 72(6):413–417
- Bowen WH (2002) Do we need to be concerned ABOUT dental caries in the coming millennium? *Crit Rev Oral Biol Med* 13(2):126–131
- Das A, Nasim I (2017) Overview of recent progress in dental application of nanozinc particles. *Pharm Educ Res J* 7:43–45
- Elgamily HM, El-Sayed HS, Abdelnabi A (2018) The antibacterial effect of two cavity disinfectants against one of cariogenic pathogen: an In vitro comparative study. *Contemp Clin Dent* 9(3):457
- Ferrando-Magraner E et al (2022) Mechanical, antibacterial, biocompatible and microleakage evaluation of glass ionomer cement modified by nanohydroxyapatite/polyhexamethylene biguanide. *Medicina* 3:56–55
- Fine DH, Furgang D, Markowitz K, Sreenivasan PK et al (2006) In vitro antibacterial activity of commonly used toothpastes in nigerian against dental pathogens. *J Am Dent Assoc* 137(10):1406–1413
- Gemert-Schricks V et al (2008) Clinical study on antibacterial activity of toothpastes against dental plaque. *Clin Oral Invest* 12:361–368
- Hirota K, Sugimoto M, Kato M, Tsukagoshi K, Tanigawa T, Sugimoto H (2010) Preparation of zinc oxide ceramics with a sustainable antibacterial activity under dark conditions. *Ceram Int* 36(2):497–506
- Inetianbor JE et al (2014) In-vitro antibacterial activity of commonly used toothpastes in nigerian against dental pathogens. *J Adv Sci Res* 5(2):40–45
- Knighton HT (1986) Antibacterial activity of four mouthrinses containing triclosan against salivary *Staphylococcus aureus*. *J Dent Res* 39:906–911
- Kolenbrander PE, Palmer RJ Jr, Rickard AH, Jakubovics NS et al (2000) Bacterial interactions and successions during plaque development. *Periodontol* 42(1):47–79
- Li YH, Lau PCY, Lee JH, Ellen RP et al (2001a) Novel two-component regulatory system involved in biofilm formation and acid resistance in streptococcus mutans. *J Bacteriol* 183(3):897–908
- Li YH, Lau PCY, Lee JH, Ellen RP et al (2001b) Natural genetic transformation of *Streptococcus mutans* growing in biofilms. *J Bacteriol* 183(3):897–908
- Loesche WJ (1986) Role of *Streptococcus mutans* in human dental decay. *Microbiol Rev* 50:353–380
- Lowy FD (1998) *Staphylococcus aureus* Infections. *N Engl J Med* 339(8):520–532
- Matsumoto-Nakano M (2018) Role of *Streptococcus mutans* surface proteins for biofilm formation. *J Dent Sci Rev* 54(1):22–29
- Newbrun E (1967) Odontologisk Revy, role of sugar and sugar substitutes in dental caries: a review. *Int Sch Res Not* 18(4):373–386
- Paster BJ, Boches SK, Galvin JL, Ericson RE et al (2001) Bacterial diversity in human subgingival plaque. *J Bacteriol* 183(12):3770–3783
- Piochi BJ, Zelante F (1974) Antibacterial activity of four mouthrinses containing triclosan against salivary *Staphylococcus aureus*. *Rev Fac Odontol Sao Paulo* 13(1):91–97
- Rasmussen JW, Martinez E, Louka P, Wingett DG (2010) Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications. *Expert Opin Drug Deliv* 7(9):1063–1077
- Rezaei R, Safaei M et al (2019) The role of nanomaterials in the treatment of diseases and their effects on the immune system. *Maced J Med Sci* 7:1884–1890
- Roberts AP, Cheah G, Ready D, Pratten J et al (2001) Genetic basis of erythromycin resistance in oral bacteria. *Antimicrob Agents Chemother* 45:2943–2946
- Rodis OM, Shimono T, Matsumura S, Hatomoto K et al (2006) In vivo acid etching effect on bacteria within caries-affected dentin. *J Am Geriatr Soc* 54:1573–1577
- Safaei M, Taran M, Jamshidi L, Imani MM, Mozaffari HR, Sharifi R, Golshah A, Moradpoor H (2020) Optimum synthesis of polyhydroxybutyrate-Co₃O₄ bionanocomposite with the highest antibacterial activity against multi-drug resistant bacteria. *Int J Biol Macromol* 158:477–485. <https://doi.org/10.1016/j.jbiomac.2020.04.017>
- Shaei F, Ashnagar A, Ghavami-Lahiji M, Naja F, Amin Marashi SM (2018) An overview of recent progress in dental applications of zinc oxide nanoparticles. *Dent Biomater* 5:510–519
- Sharan S et al (2017) Applications of nanomaterials in dental science: a review. *J Nanosci Nanotechnol* 17:2235–2255
- Sirekhatim A, Mahmud S, Seeni A, Kaus NHM, Ann LC, Bakhori SKM, Hasan H, Mohamad D (2015) Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. *Nano-Micro Lett* 7:219–242
- Sutherland IW (2001) The biofilm matrix—an immobilized but dynamic microbial environment. *Trends Microbiol* 9(5):222–227
- Yoo SY, Park SJ, Jeing DK, Kim KW et al (2007) Antimicrobial properties of biofunctionalized silver nanoparticles on clinical isolates of streptococcus mutans and its serotypes. *J Microbiol* 45(3):246–255

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