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ORIGINAL ARTICLE

Comparative Evaluation of Antimicrobial Efficacy of Silver, Titanium Dioxide and Zinc Oxide Nanoparticles against Streptococcus mutans

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Abstract

Objective: To determine the antimicrobial efficacy of silver, titanium dioxide and zinc oxide nanoparticles against Streptococcus mutans. **Material and Methods:** Serial dilution method was employed for preparing 1%, 0.5%, 0.25% concentrations of the three test compounds. ATCC 25175 strain of streptococcus mutans was used to assess the antimicrobial activity of test compounds. Equal quantity of BHI broth was dispensed in test tubes containing sectioned tooth and to this the prepared bacterial inoculum was added. Prepared concentrations of test compounds were added accordingly and incubated for 24hrs at 37^{0}C. Then, the sectioned tooth was removed and the adherent bacteria were transferred into saline solution by vortexing. These suspensions were transferred onto sterile blood agar plate to make lawn culture and were further incubated at 37^{0}C for 24hrs to determine viable bacterial count. The number of colonies were counted manually from each plate and recorded for further analysis. Decrease in number of colonies represents the effective concentration of the test compound against the inhibition of biofilm formation. **Results:** A significant difference in the colony forming units among all three concentrations of silver (Ag), titanium dioxide (TiO_2) and zinc (ZnO) nanoparticles was noted and the antimicrobial effect of nanoparticles was concentration dependent. Inter group comparison of colony forming units with 1%, 0.5% and 0.25% of the test compound revealed that the colony forming units on the ZnO nanoparticles demonstrated highest value followed by TiO_2 and the least were with that of the Ag nano particles. **Conclusion:** Silver, Zinc oxide and Titanium dioxide showed significant antimicrobial effects and the antimicrobial effect of nanoparticles was concentration dependent.

Keywords: Nanoparticles; Orthodontics; Microbiological Techniques.

Introduction

Demineralization of enamel adjacent to orthodontic brackets and bands is a complication confronted by every dental practitioner and is a potential risk for individuals who do not maintain their hygiene adequately [1]. As maintaining the adequate oral hygiene becomes more difficult in these individuals, the decalcification of the enamel surface around the appliances is prevalent [2]. Individuals who are undergoing orthodontic treatment are known to be at increased risk for developing dental caries because of enhanced salivary and plaque levels associated with Streptococcus mutans. With elevated levels of bacteria and meagre oral hygiene maintenance, decalcifications can ensue in as little as in one month [3-5].

Application of fluoride varnishes, use of fluoride mouthwash, and meticulous oral hygiene instructions have been employed to counter the demineralisation. These techniques depend for the most part on the consistence of the patient and they give just periodic aegis against decalcification [6].

Nanotechnology has been used in dentistry to provide enhanced mechanical properties and antibacterial effects. These materials contribute for remarkable antimicrobial properties and demonstrate comparable physical properties when compared with conventional materials because of their small size and increased surface area [7,8].

Numerous antibacterial agents have been integrated into orthodontic products and authorized for intraoral applications. It has been reported that Streptococcus mutans is sensitive to Ag, ZnO and Au, which would permit accomplishing vital clinical effects [9]. Because of its antimicrobial activity, Ag has a long history of utilization in medical field. Reduction in the adhesion of cariogenic streptococci to orthodontic adhesives is observed by incorporating Ag nanoparticles into composite resins [10]. Previous studies have indicated that TiO_2 nano-particles exhibit significant antimicrobial effects [11]. ZnO powders also display antimicrobial efficacy and shown to kill several oral microbes known to contribute to caries [12,13]. With this background the present study has aimed at the determination of the antimicrobial efficacy of nanoparticles of Ag, ZnO and TiO_2 against Streptococcus mutans.

Material and Methods

Microorganism

Lyophilized culture of Streptococcus mutans (ATCC 25175)

Preparation of Culture Broth

Lyophilized culture of Streptococcus mutans (ATCC 25175) was reconstituted by inoculating into brain heart infusion (BHI) broth and was incubated at 37°C to obtain an overnight broth culture.

Preparation of Blood Agar Plates

Blood agar media was prepared by aseptical addition of 10% sheep blood to autoclaved nutrient agar at a temperature of 45°C and allowed to solidify after transferring into sterile petri plates. A loop full of inoculum from overnight broth culture of Streptococcus mutans is streaked onto the well dried blood agar plate. This is incubated at 37°C for 24hrs to obtain fresh growth of Streptococcus mutans. Few colonies from this blood agar culture were emulsified into a sterile normal saline using a sterile loop to make a uniform suspension. The density of the bacterial suspension was adjusted with sterile phosphate buffer saline (PBS) to match the density of McFarland standard 0.5.

Serial Dilution Method

Equal quantity of 160 mg of Ag, TiO_2, ZnO nano particles were weighed and stored in a sterile container. These were added to three different test tubes containing 4ml of BHI broth which represents 4% concentration of the compound (10mg of test compound in 1ml BHI broth = 1% of test compound).

Serial Dilution Method for Ag Nanoparticles Group

Three test tubes containing 2ml of BHI broth were obtained. To these test tubes sectioned tooth was added and the tubes are labelled as 1%, 0.5% and 0.25% which denote the amount of test compound within each group. 1ml of the above mixed solution is now transferred into the second tube labelled as 1%. Following the transfer of the compound, the test tube is vortexed to allow for complete mixing of the compound with the BHI broth. The same technique was done for, 0.5% & 0.25%. 2ml from the last tube (0.25%) was discarded and thus all the test tubes contained the respective concentration of the test compound with equal quantity (2ml) of BHI broth.

Similar method was employed for all the other test compounds (TiO_2 and ZnO). A drop of standard bacterial suspension is added using a sterile dropper to all the test tubes.

Determination of Efficacy against the Inhibition of Streptococcus mutans Formation

The tooth from each group was transferred separately into the sterile tubes containing 1ml of sterile normal saline after an incubation period of 48hrs. All the tubes were vortexed for 2minutes to allow for the detachment of the adherent bacteria from the tooth. The vortexed saline suspension is then transferred to the sterile blood agar plates to make lawn culture and was further incubated at 37°C for 24 hrs to determine the viable count of bacteria. The number of colonies from each plate were counted manually and recorded for further analysis. These colonies represent the number of bacteria that were adhered on the surface of tooth. Test groups with differing concentrations were compared for statistical analysis. Decrease in the number of colonies indicates the efficacy of test compound (Ag, TiO_2, ZnO nanoparticles) on the inhibition of Streptococcus mutans formation on the enamel surface of tooth.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics for Windows Software, version 22 (IBM Corp., Armonk, NY, USA). Descriptive statistics was used to calculate the minimum and maximum values, mean and standard deviation. The comparison between two or more groups was done by one way analysis of variance test and followed Bonefferini's post-hoc test. All p-values less than 0.05 were statistically significant.

Results

Table 1 demonstrates a statistically significant difference in the colony forming units among all the three concentrations used with silver, titanium dioxide and zinc nanoparticles ($p<0.0001$) and the antimicrobial effect of the nanoparticles was concentration dependent.

Table 1. Distribution of minimum, maximum, mean, and standard deviation (SD) values.

Group	N	Minimum	Maximum	Mean	SD	p-Value
Silver						
1%	5	320	600	454	105.3	
0.50%	5	978	1000	992	9.975	<0.0001
0.25%	5	9850	10000	9940	73.82	
Titanium Dioxide						
1%	5	750	1000	898	107.3	
0.50%	5	1000	1500	1300	203.1	<0.0001
0.25%	5	8950	10000	9692	458.4	
Zinc						
1%	5	980	1200	1054	94.8	
0.50%	5	8300	9900	8940	634.8	<0.0001
0.25%	5	9540	10000	9854	185.7	

Inter group comparison of colony forming units with 1%, 0.5% and 0.25% of all the three test compound revealed that the colony forming units on the ZnO nanoparticles demonstrated highest value followed by TiO_2 % and the least were with that of the Ag nano particles (Table 2).

Table 2. The comparison of Colony Forming Units between the groups.

		Colony Forming Units (CFU)				
Groups	**N**	**Minimum**	**Maximum**	**Mean**	**SD**	**p-value**
		1% Test Compound				
Ag 1%	5	320	600	454	105.3	
TiO_2 1%	5	750	1000	898	107.3	<0.0001
ZnO 1%	5	980	1200	1054	94.76	
		0.5% Test Compound				
Ag 0.5%	5	978	1000	992	9.975	
TiO_2 0.5%	5	1000	1500	1300	203.1	<0.0001
ZnO 0.5%	5	8300	9900	8940	634.8	
		0.25% Test Compound				
Ag 0.25%	5	9850	10000	9940	73.82	
TiO_2 0.25%	5	8950	10000	9692	458.4	0.414
ZnO 0.25%	5	9540	10000	9854	185.7	

Pairwise comparisons within the subgroup 1% were made to evaluate the mean difference. A significant differences were observed between 1% Ag *versus* 1% ZnO and with 1% Ag *versus* 1% TiO_2 but no statistical difference was seen between 1% TiO_2 *versus* 1% ZnO (Table 3). The comparisons within the subgroup 0.5% revealed significant differences between all the nanoparticles (Table 3) and pairwise comparisons within the subgroup 0.25% demonstrated no significant differences were between all the nanoparticles (Table 3).

Table 3. Comparison of Colony Forming Units (CFU) with different percentage of test compound.

Comparisons	p-value
1% Test Compound	
Ag 1% vs TiO_2 1%	0.000
Ag 1% vs ZnO 1%	0.000
TiO_2 1% vs ZnO 1%	0.014
0.5% Test Compound	
Ag 0.5% vs TiO_2 0.5%	0.003
Ag 0.5% vs ZnO 0.5%	0.000
TiO_2 0.5% vs ZnO 0.5%	0.000
0.25% Test Compound	
Ag 0.25% vs TiO_2 0.25%	0.089
Ag 0.25% vs ZnO 0.25%	0.121
TiO_2 0.25% vs ZnO 0.25%	0.162

Discussion

Different nanoparticles have attained huge enthusiasm throughout the years because of their notable antimicrobial properties. The incredible antibacterial impact of these nanostructured specialists is fundamentally ascribed to the increased surface area to volume ratio empowering more noteworthy latency of atoms on the surface, which provides maximum contact with the environment [14,15]. Likewise, the minimal size of nanoparticles enables penetration through cell membranes simpler, hence influencing intracellular processes bringing about enhanced reactivity and antimicrobial property [16].

It has been reported that Ag nanoparticles have antibacterial property against Streptococcus mutans and inhibits its growth [16]. Previous authors noted that orthodontic composite adhesives comprising of nanosilver fillers particles prevents enamel demineralization around bracket surfaces without arbitrating their physical properties [17].

In the present study 1% nanosilver content revealed highest antibacterial effect, similar to previous report [18]. Whereas another authors observed that 5% Ag/HA nanoparticles limits the growth of cariogenic bacteria and increasing the concentration of Ag/HA nanoparticles did not reveal any compelling curtailment [19].

In spite of the fact that the exact antimicrobial mechanism of silver nanoparticles has not established, it has been proposed that catalytic action of silver converts the oxygen to active oxygen

by light energy in the air or water, since silver ion can aid electron deracination from a molecule. The active oxygen thus produced leads to structural damage in microbes, denaturation of proteins and enzymes of bacteria [20].

ZnO nanoparticles possess a broad range of antibacterial spectrum and can kill organisms, which cause caries [21]. These nanoparticles have scrupulous toxicity to bacteria, minimally affecting the human cells [22]. In the present study ZnO showed significant antimicrobial effects and the antimicrobial effect of the ZnO was concentration dependent. These findings agree with other studies [23,24]. It has been proposed that the antimicrobial action is due to blocking action of zinc on the electron-transport chain or by limiting the generation of ATP. Zinc is also known to hinder with transport activity by selectively blocking the membrane locations leading to conformational alterations in proteins and enzymes [25]. ZnO nanoparticles also known to act by generating reactive active oxygen species such as H_2O_2, which are known to hinder the growth of planktonic microbes [21].

TiO_2 nano-particles are available in varying dimensions and crystalline structures and are recommended as suitable for adding into various dental materials. These nanoparticles are comparatively economical with exemplary mechanical properties and adorable color. The results of the present study revealed that 1% TiO_2 nanoparticles (w/w) could significantly reduce bacterial growth and this finding was in accordance with previous observations [26,27].

Even though the employment of nanoparticles in the orthodontics speciality is picking up quality, a few confinements and disadvantages have likewise been additionally reported. Ag nanoparticles are thought to bring about a dark gray discoloration and are recommended as incompatible and not appropriate for use in dentistry. Zn based nanoparticles are thought to deliver extreme toxic impacts in animal studies [9].

No matter their ambiguous antimicrobial competency, incorporating the nanoparticles has been asserted to be inadmissible and also known to compromise the mechanical properties [26]. In agreement with this, our previous research revealed that incorporation of various nanoparticles into adhesive materials in minimal amounts can compromise the shear bond strength causing the failure of bracket or adhesive [28].

Conclusion

Silver (Ag), zinc oxide (ZnO) and titanium dioxide (TiO_2) showed significant antimicrobial effects. From the results of our study, it has also been concluded that as the concentration of the nanoparticles increases, antimicrobial activity significantly increases.

References

1. Gorelick L, Geiger AM, Gwinnett AJ. Incidence of white spot formation after bonding and banding. Am J Orthod Dentofacial Orthop 1982; 81(2):93-8.

2. Enaia M, Bock N, Ruf S. White-spot lesions during multibracket appliance treatment: A challenge for clinical excellence. Am J Orthod Dentofacial Orthop 2011; 140(1):e17-24. doi: 10.1016/j.ajodo.2010.12.016.

3. Guzmán-Armstrong S, Chalmers J, Warren JJ. Ask us. White spot lesions: prevention and treatment. Am J Orthod Dentofacial Orthop 2010; 138(6):690-6. doi: 10.1016/j.ajodo.2010.07.007.
4. Chen W, Zhou Y. Caries outcomes after orthodontic treatment with fixed appliances: A longitudinal prospective study. Int J Clin Exp Med 2015; 8(2):2815-22.
5. Srivastava K, Tikku T, Khanna R, Sachan K. Risk factors and management of white spot lesions in orthodontics. J Orthod Sci 2013; 2(2):43-9. doi: 10.4103/2278-0203.115081.
6. Lopatiene K, Borisovaite M, Lapenaite E. Prevention and treatment of white spot lesions during and after treatment with fixed orthodontic appliances: A systematic literature review. J Oral Maxillofac Res 2016; 7(2):e1. doi: 10.5037/jomr.2016.7201.
7. Ozak ST, Ozkan P. Nanotechnology and dentistry. Eur J Dent 2013; 7(1):145-51.
8. Abou Neel EA, Bozec L, Perez RA, Kim H-W, Knowles JC. Nanotechnology in dentistry: Prevention, diagnosis, and therapy. Int J Nanomedicine 2015; 10:6371-94. doi: 10.2147/IJN.S86033.
9. Hernández-Sierra JF, Ruiz F, Pena DC, Martínez-Gutiérrez F, Martínez AE, Guillén Ade J, Tapia-Pérez H, Castañón GM. The antimicrobial sensitivity of Streptococcus mutans to nanoparticles of silver, zinc oxide, and gold. Nanomedicine 2008; 4(3):237-40. doi: 10.1016/j.nano.2008.04.005.
10. Holla G, Yeluri R, Munshi AK. Evaluation of minimum inhibitory and minimum bactericidal concentration of nano-silver base inorganic anti-microbial agent (Novaron®) against streptococcus mutans. Contemp Clin Dent 2012; 3(3):288-93. doi: 10.4103/0976-237X.103620.
11. Kasraei S, Sami L, Hendi S, AliKhani M-Y, Rezaei-Soufi L, Khamverdi Z. Antibacterial properties of composite resins incorporating silver and zinc oxide nanoparticles on Streptococcus mutans and Lactobacillus. Restor Dent Endod 2014; 39(2):109-14. doi: 10.5395/rde.2014.39.2.109.
12. Elsaka SE, Hamouda IM, Swain MV. Titanium dioxide nanoparticles addition to a conventional glass-ionomer restorative: Influence on physical and antibacterial properties. J Dent 2011; 39(9):589-98. doi: 10.1016/j.jdent.2011.05.006.
13. Palanikumar L, Ramasamy SN, Balachandran C. Size-dependent antimicrobial response of zinc oxide nanoparticles. IET Nanobiotechnol 2014; 8(2):111-7. doi: 10.1049/iet-nbt.2012.0008.
14. Hajipour MJ, Fromm KM, Ashkarran AA, Jimenez de Aberasturi D, de Larramendi IR, Rojo T, et al. Antibacterial properties of nanoparticles. Trends Biotechnol 2012; 30:499-510. doi: 10.1016/j.tibtech.2012.06.004.
15. Park HJ, Park S, Roh J, Kim S, Choi K, Yi J, Kim Y, Yoon J. Biofilm-inactivating activity of silver nanoparticles: A comparison with silver ions. J Ind Eng Chem 2013; 19(2):614-9. doi: 10.1016/j.jiec.2012.09.013.
16. Degrazia FW, Leitune VCB, Garcia IM, Arthur RA, Samuel SMW, Collares FM. Effect of silver nanoparticles on the physicochemical and antimicrobial properties of an orthodontic adhesive. J Appl Oral Sci 2016; 24(4):404-10. doi: 10.1590/1678-775720160154.
17. Ahn SJ, Lee SJ, Kook JK, Lim BS. Experimental antimicrobial orthodontic adhesives using nanofillers and silver nanoparticles. Dent Mater 2009; 25(2):206-13. doi: 10.1016/j.dental.2008.06.002.
18. Miresmaeili A, Atai M, Mansouri K, Farhadian N. Effect of nanosilver incorporation on antibacterial properties and bracket bond strength of composite resin. Iran J Orthod 2012; 7:14-19.
19. Sodagar A, Akhavan A, Hashemi E, Arab S, Pourhajibagher M, Sodagar K, et al. Evaluation of the antibacterial activity of a conventional orthodontic composite containing silver/hydroxyapatite nanoparticles. Prog Orthod 2016; 17:40. doi: 10.1186/s40510-016-0153-x.
20. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv 2009; 27(1):76-83. doi: 10.1016/j.biotechadv.2008.09.002.
21. Sevinç BA, Hanley L. Antibacterial activity of dental composites containing zinc oxide nanoparticles. J Biomed Mater Res B Appl Biomater 2010; 94(1):22-31. doi: 10.1002/jbm.b.31620.
22. Jones N, Ray B, Ranjit KD, Manna AC. Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. FEMS Microbiol Lett 2008; 279(1):71-6. doi: 10.1111/j.1574-6968.2007.01012.x.
23. Spencer CG, Campbell PM, Buschang PH, Cai J, Honeyman AL. Antimicrobial effects of zinc oxide in an orthodontic bonding agent. Angle Orthod 2009; 79(2):317-22. doi: 10.2319/011408-19.1
24. Moorer W, Genet J. Antibacterial activity of gutta-percha cones attributed to the zinc oxide component. Oral Surg Oral Med Oral Pathol 1982; 53(5):508-17.
25. Bates D, Navia J. Chemotherapeutic effect of zinc on Streptococcus mutans and rat dental caries. Arch Oral Biol 1979; 24(10-11):799-805. doi: 10.1016/0030-4220(82)90468-6.

26. Elsaka SE, Hamouda IM, Swain MV. Titanium dioxide nanoparticles addition to a conventional glass-ionomer restorative: influence on physical and antibacterial properties. J Dent 2011; 39(9):589-98. doi: 10.1016/j.jdent.2011.05.006.

27. Poosti M, Ramazanzadeh B, Zebarjad M, Javadzadeh P, Naderinasab M, Shakeri MT. Shear bond strength and antibacterial effects of orthodontic composite containing TiO2 nanoparticles. Eur J Orthod 2013; 35(5):676-9. doi: 10.1093/ejo/cjs073.

28. Reddy AK, Kambalyal PB, Patil SR, Vankhre M, Khan MY, Kumar TR. Comparative evaluation and influence on shear bond strength of incorporating silver, zinc oxide, and titanium dioxide nanoparticles in orthodontic adhesive. J Orthodont Sci 2016; 5(4):127-31. doi: 10.4103/2278-0203.192115.