Antimicrobial efficacy of silver, zinc oxide, and titanium dioxide nanoparticles incorporated in orthodontic bonding agent

Sarah Yousif Hailan B.D.S. [(1)]
Mustafa M. Al-Khatieeb B.D.S., M.Sc. (Orthodontics) [(2)]

ABSTRAT

Background: One of the most important complications of fixed orthodontic treatment is formation of white spots, which are initial carious lesions. Addition of antimicrobial agents into orthodontic adhesive material might be wise solution for prevention of white spots formation. The aim of this study was to evaluate the antibacterial properties of orthodontic adhesive primer against *S. Mutans* after adding the three different types of nanoparticles (Ag, ZnO, or TiO_2).

Materials and methods: Discs were prepared using empty insulin syringe approximately 2 mm×2 mm rounded in shape specimens (40 discs) were divided into four groups (ten discs for each group): The first group was the control (made from primer only), the second group (10 discs made from primer and incorporation of Ag NPs), the third group (10 discs made from primer and incorporation of ZnO NPs), and the fourth group (10 discs made from primer and incorporation of TiO_2 NPs). The antibacterial properties of orthodontic bonding agent after incorporation of (Ag, ZnO, or TiO_2) nanoparticles were evaluated by disc diffusion test and viable count of *mutans streptococci.*

Results: The results of this study showed that there were high significant differences between the all groups using ANOVA F-test, and the colony forming unit were 99×10³, 39.6×10³, 19.4×10³, 6.6×10³ CFU/mL respectively. Conclusion: The incorporation of these nanoparticles (Ag, ZnO, or TiO_2) into Transbond ™ XT adhesive primer helps to enhance the antibacterial properties of primer against the *S. mutans.*

Keywords: *Mutans streptococci*, Nanoparticles, Antimicrobial activity, Silver, Zinc oxide, Titanium dioxide. **(Received: .29/11/2018; Accepted: 2/1/2019)**.

INTRODUCTION

Both the brackets and bonding adhesive materials may retain plaque as a result of this new site is susceptible to caries, because of the level of *Streptococcus mutans* is significantly increased in the saliva and plaque of patients who are undergoing to fixed orthodontic appliance treatment, and the result is elevated risk of the caries [(1,2)]. Nanofillers can minimize enamel demineralization with no deterioration of physical properties of the composite [(3)]. Researchers confirmed that new adhesive system composed of silver nanoparticles (Ag) provided an excellent antibacterial properties [(4)]. Zinc-based nanoparticles are stated to bring persistent harmful effects in animal studies *in vitro* [(5)]. Several studies have reported that resins containing TiO_2 nanoparticles show antimicrobial properties which may be applied for preventing frequent caries and demineralization of the enamel [(6-8)]. The silver, zinc oxide, and TiO_2 have proper antibacterial activity and when they convert into nanoparticles their surface to volume ratio increases, and this will improving their antibacterial activity [(9-11)].

(1)Master student, Department of Orthodontics, College of Dentistry, University of Baghdad.
(2)Assistant Professor, Department of Orthodontics, College of Dentistry, University of Baghdad.

MATERIALS AND METHODS

Adhesive preparation:

The primer of the first group was left without any additives (control), while the 2nd group, 1% silver nanoparticles (80nm, purity 99%) were incorporated to the primer [(12-15)], the 3rd group, 1% zinc oxide nanoparticles (50nm, purity 99%) were incorporated to the primer [(14,16,17)], and 4th group, 1% titanium dioxide nanoparticles (25nm, purity 99%) were incorporated to the primer [(7,8,14,18)], each mixture (a primer with one type of nanoparticle) was placed in a test tube covered with foil and mixed for 2 min using Vortex machine (Huma Twist, Wiesbaden, Germany) to create uniform homogenous mixture (Figure 1).

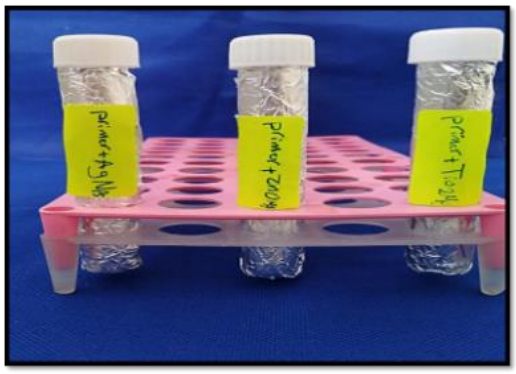

Figure 1: The primer agents after adding the nanoparticles.

Discs preparation

Discs were prepared using empty insulin syringe (1mL) (AMART, New Delhi, India), the syringe sliced using hand piece at 3200 RPM to form disc that are approximately 2mm×2mm rounded in shape specimens, 40 discs were prepared and divided into four groups (one control group, and three test groups according to the type of nanoparticles which had incorporated), and four bonding mixture groups added to the discs separately (ten discs for each group) and light cured for 40 seconds, which are the control group with primer only without adding NPs, and three experimental groups which were 1% Ag NPs, 1% ZnO NPs, and 1% TiO_2 NPs. After setting these discs kept in sterilized containers until antimicrobial test [19]. Figure 2.

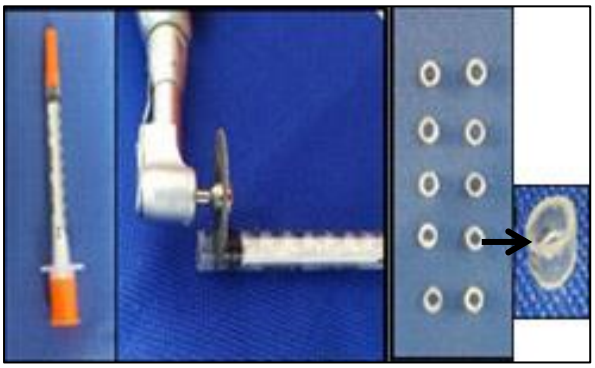

Figure 2: A. Insulin empty syringe 1Ml; B. Slicing by hand piece; C. The mold obtained.

Agar plate preparation: *Mitis- Salivarius* Bacitracin Agar (MSB agar)

This agar is the selective medium for the *mutans streptococci*. It was prepared from *mitis salivarius* agar (MSA) according to Hi-Media Company instructions with 20% (W/V) sucrose and 200I.U/L bacitracin [20,21]. The preparation of media was done according to the instructions of manufacturer by suspending 90gm of the powder in 1000mL distilled water, mixed well by Magnetic stirrer (Zhongxing, Taiwan, China) to ensure dissolution of the entire quantity of the powder, to increase the specialty of MSB agar to the isolation of *Mutans streptococcus*, before sterilization, the addition of sucrose in a concentration of 150 gm/L was done. The medium was cool to about 45°C after autoclaving at 121°C for 15 min under 15 psi then left to cool till 45-50°C after that 1mL of bacitracin solution was added for each one liter of the agar. The preparation of bacitracin stock solution was done by dissolving 0.364g of powder in 100mL of sterilized distilled water mixed well by Magnetic stirrer to make sure dissolution of the whole quantity of the antibiotic, this will provide concentration of 200 I.U/L. The solution was pure by millipore filter (0.20µm) (Fisher scientific, Wien, Austria) until use, it will be kept in refrigerator. A new solution was prepared each two weeks [20,22], then it was poured in petri dishes and allowed to cool and set then stored in the refrigerator (Samsung, South Korea) at 2-8°C until used, according to the instruction of Hi Media company [23]. Figure 3.

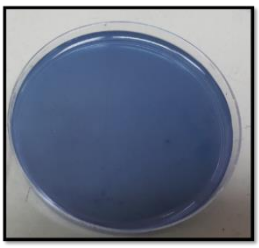

Figure 3: *Mitis- Salivarius* Bacitracin Agar Blood agar.

The preparation of media was done according to the instructions of Hi Media company by adding 40 gm in 1000ml distilled water, heated to the boiling temperature at 80°C to dissolve the medium completely, sterilized by autoclaving at 15 psi 121°C for 15 min, then cooled to 45-50 °C, and aseptically added 5% V/V defibrinated blood, furthermore, mixed well and poured into sterile Petri dish [22,23].

Identification of *Mutans streptococci*

A. Morphological characteristics:

Mutans streptococci were examined under light microscope (magnification X 15) (Olympus, Tokyo, Japan), the colonies appear light blue in color about 1-2 mm in diameter as spherical or ovoid in shape with raised or convex surface, adhered well to the agar surface. Most of *Mutans streptococci* colonies has a depression at the middle of the colony containing a drop of polysaccharide, or sometimes the whole colony submerged in a pool of polysaccharide [24,25].

B. Gram's stain

One or two colonies were selected from MSB agar under sterilized condition and subjected to Gram's stain (Vaccine and Sera Institute, Hadapsar, India) [26].

C. Biochemical tests

The following tests were conducted:

1- Catalase production test

A small amount of pure isolates of *Mutans streptococci* were transferred individually using a sterile loop to the surface of clean dry glass slide. Drops of hydrogen peroxide 3% (Gemtek, New Jersey, USA)

directly onto a portion of bacterial culture on the slide (Citotest, Nantong, China), the absence of catalase enzyme indicated by absence of gas bubbles, this test can be done also on the colonies of MSB agar plates directly [27].

2- Carbohydrate fermentation test for *Mutans streptococci*

In order to evaluate the ability of MS to ferment the mannitol, Cystine Trypticase - Mannitol Agar had been used, the mannitol was added in a concentration of 1% to the Cystine Trypticase - Agar and spread into screw capped bottles (10mL in each bottle) and sterilized by autoclave, then kept in the refrigerator at 2-8ºC until used. Each bottle was inoculated with 0.1mL of pure MS isolates and incubated aerobically at 37ºC for 48 hours. The color will be changed from red to yellow that means there is a positive reaction as a result of acid production from the fermentation reaction [28].

The antibacterial activity assay:

A. Determining the antimicrobial activity of different nanoparticles on the *mutans streptococci* growth group:

Kirby- Baure disk diffusion test was performed. Three or four well isolated colonies of *mutans streptococci* from MSB agar were suspended in 5ml sterile normal saline to achieve 0.5 McFarland turbidity to produce a stock suspension [29]**.** A sterile swab was immersed into this suspension and excess fluid was pushed out. The brain heart agar was swabbed carefully in three directions to achieve uniform growth on the surface of the agar plate [30]**.** After the agar surface has been left for about five min, then the discs (with and without NPs) were placed on the agar and the plates were kept at room temperature for 120 min for diffusion of the antimicrobial agents [31]**,** then these agar plates were incubated aerobically at 37ºC for 24 hours. In order to measure the inhibition zone that may appear around the discs, a polygauge caliper was used [29]. Figure (4).

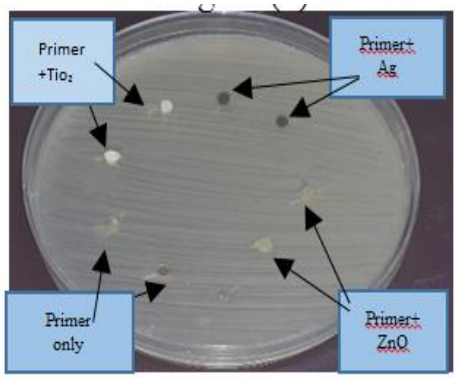

Figure 4: The discs placed inside the BHI agar

B. The effect of nanoparticle loaded resins on viable count of *mutans streptococci* group colonies:

To examine the antimicrobial activity of the primer with Ag, ZnO, or TiO_2 Nps. *mutans streptococci* was diluted in 0.9% NaCl, and suspension of approximately 1.5×10^8 CFU/mL, a 0.5 McFarland standards was prepared using a McFarland densitometer**,** this was done by transferring 1-2 colonies of *streptococci mutans* from 24 hours cultures of blood agar to obtain the suspension [32,33]. By micropipette 1000μL of prepared bacterial suspension was inoculated into 9mL of Brain-Heart infusion broth under sterile condition [34], and serially dilution was done by adding 1000μL to the second tube of 9ml of BHI-broth, then adding 1000μL from the second tube to the third one 10^{-3} (BHI-broth with 9mL), and the sterile discs that were going to be examined placed individually into the inoculated tubes, and incubated at 37ºC for 1 hour under aerobic condition, after that each disc removed from tubes and placed in 5mL normal saline, and shacked by Vortex mixer for 2 min to allow releasing of bacteria from the specimen surface, then 100µl from this solution was taken by micropipette and spread on blood agar, and incubated aerobically for 24 hours at 37ºC [35-38]. The final count (number of bacteria per milliliter) was calculated in the following equation:

Colony forming unit per mL (CFU/mL) = Colony number/ Dilution Factor [36,37]. Figure 5.

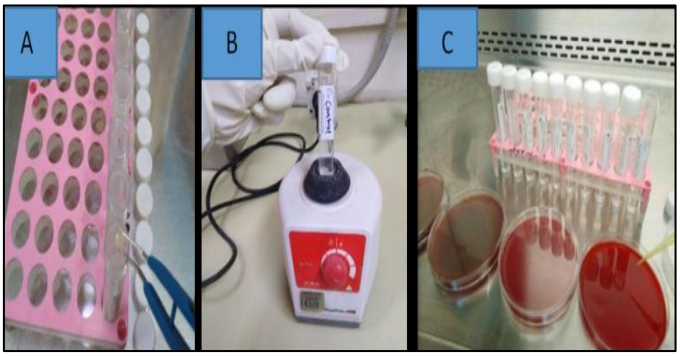

Figure 5: A. Placement of specimen in the normal saline; B. Shacked by auto Vortex for 2 min; C. Add 100 µl to the blood agar

Statistical Analysis

All statistical tests and calculations were made using Statistical Package for Social Science software (SPSS for windows, 19.0, Chicago, USA). Testing the normality of data distribution was carried out by using Shapiro-Wilk test. Maximum, minimum, mean values, standard error and standard deviations were

calculated as part of the descriptive analysis. Statistical significances were measured using one way (ANOVA) to discover the difference among the four groups, and Post hoc Tukey's test used to test further any statistically significant difference between each two groups.

RESULTS

Evaluating viable count of SM (CFU/mL)

Testing the normality of data distribution was carried out by using Shapiro-Wilk test, in order to apply the correspondence statistical test. The results showed that there was no significant difference in all the groups of antimicrobial test; as shown in Table1, while descriptive statistics and F-test analysis of variance (ANOVA) of mean values of CFU/mL in different groups were evaluated, there was high significant difference among the groups as shown in Table 2, also Post hoc Tukey's test was used to test the mean differences and showed that there were high significant differences in CFU/mL between the control group and all tested groups; as shown in Table 3.

Table 1: Testing the normality of data distribution for the antimicrobial test in the various groups.

Groups	Shapiro-Wilk	d.f.	p-value
Control	0.940	10	0.554 (NS)
Ag	0.941	10	0.562 (NS)
ZnO	0.918	10	0.345 (NS)
TiO_2	0.952	10	0.692 (NS)

NS: Non significant

Table 2: Descriptive statistics and F-test (ANOVA) of the CFU/mL.

Descriptive statistics							Comparison (df=39)	
Groups	**N**	**Mean**	**S.D.**	**S.E.**	**Min.**	**Max.**	**F –test d.f=39**	**p-value**
Control	10	99×10^3	7.57	2.39	89	110	346.873	0.001 (HS)
Ag	10	39.6×10^3	10.28	3.25	26	56		
ZnO	10	19.4×10^3	4.93	1.56	14	30		
TiO_2	10	6.6×10^3	2.37	0.75	3	10		

HS: Highly significant

Table 3: Post hoc Tukey's test of the CFU/mL in different groups.

Groups		Mean Difference	p-value
Control	Ag	59.4×10^3	0.001 (HS)
	ZnO	79.6×10^3	0.001 (HS)
	TiO_2	92.4×10^3	0.001 (HS)
Ag	ZnO	20.2×10^3	0.001 (HS)
	TiO_2	33×10^3	0.001 (HS)
ZnO	TiO_2	12.8×10^3	0.001 (HS)

HS: Highly significant

DISCUSSION

Identification of *mutans streptococci*

A. Colony morphology

MS colonies appeared light blue in color, about 1-2 mm in diameter as spherical or ovoid in shape with raised or convex surface, adhered well to the *mitis salivarius* agar surface, this media has selective and differential properties because it contained Bacitracin which inhibits the growth of other type of bacteria [39].

B. Gram stain test

The Gram stain was used because it was the most useful and widely employed differential stain in bacteriology. The MS bacteria was gram positive, which stained with the basic dye crystal

violet, this is the primary stain, it is followed by treatment with an iodine solution, which functions as a mordant; it increases the interaction between the bacterial cell and the dye, so that the dye is more tightly bound or the cell is more strongly stained [36].

C. Biochemical tests

1. Catalase production test

This test demonstrates the presence of catalase enzyme, it was used to differentiate bacteria that produce an enzyme catalase or not. The enzyme catalase facilitates the breakdown of hydrogen peroxide into oxygen and water. The presence of the enzyme in a bacterial isolate was evident when the rapid elaboration of oxygen bubbles occurs. The lack of catalase enzyme was evident by a lack of or weak bubble production [40].

2. Carbohydrate fermentation test

About 1% of specific carbohydrate (which was one of components of Cystine Trypticase Manitol Agar) was used to detect fermentation reactions. Changing the color of the indicator (Phenol red) from red to yellow which points the fermentation of carbohydrate and acid production [41-43].

Disc diffusion test and plate counted method

In some studies, diffusion agar disc technique has been used to evaluate antibacterial properties [44-46]. In the current study, the adhesive materials do not form the growth inhibitory zone, the fact behind that is the direct contact of the nanoparticles that were added to the primer of the adhesive with bacteria is minimal [9,44,46]. However, turbidity measurements are limited, as they count both dead and vital bacteria present together in a biofilm layer [47]. The contact area of nanoparticles with bacterial micro-organisms is higher in a serial dilution method compared to the culture media, thus increasing their antibacterial effect [11]. Therefore, the obtained results of this study are depend on plate count method in which a viable cell count allows one to identify the number of actively growing cells in a sample, the plate count method or spread plate relies on bacteria growing ability to form a colony on a nutrient medium, the colony becomes visible to the naked eye and the number of colony on a plate can be counted [36]. The results of the present study showed that the number of colonies of viable bacteria of *Streptococcus mutans* in the control group was significantly higher than that in the three other tested groups after incorporating the nanoparticles (Ag, ZnO, or TiO_2) to the Transbond XT primer because these types of nanoparticles provide an excellent antibacterial activities [4-8], and this agreed with researchers [7,12]. A highly significant difference was found between Ag group and the control group, this may be due to high antibacterial properties of Ag NPs, this result agreed with other studies [8,10-12,48]. A highly significant difference was found between ZnO group and the control group, this may be due to the ZnO NPs serves as an activator of enzymes that can be toxic to bacteria, this result agreed with other studies [11,12,46,49]. A highly significant difference was found between TiO_2 group and the control group, and this may be due to TiO_2 NPs have a broad spectrum antimicrobial agent, this result agreed with [7,8,48,49]. In addition there is a highly significant difference between Ag group and ZnO group (antimicrobial activity of ZnO group was higher than Ag group), this may be due to the particle size of ZnO NPs relative smaller than the size of Ag NPs, this result agreed with researchers [12,49], but contrary to the result of others [11], in which they evaluated the effects of silver (25 nm), and zinc oxide (125 nm) nanoparticles on *Streptococcus mutans* and reported that the antibacterial activity of silver nanoparticles is much higher than those of zinc oxide and gold nanoparticles, these differences might be attributed to the size of the applied nanoparticles. Furthermore, our study showed that there were a highly significant differences between TiO_2 and Ag groups, and TiO_2 and ZnO groups (antimicrobial activity of TiO_2 group was higher than Ag and ZnO groups), this may be due to the size of TiO_2 NPs relative smaller than the size of Ag and ZnO NPs, these results in accordance with a study [49], but disagreed with another [48], who found that the antimicrobial effect of Ag nanoparticles on MS was more than that of TiO_2 nanoparticles, this may be attributed to the difference in size of applied nanoparticles that was used in their studies. The antimicrobial activity of Ag, ZnO, and TiO_2 NPs appeared to be particle size dependent, the smaller particle size will lead to efficient ions release due to their large surface-to-volume ratio that could enable them to discharge more ions at a low concentration, this may result in defect in bacterial cell wall, so that the cell contents are lost and this result can agree with other studies [10,11,46] . In the current study, the incorporation of these nanoparticles (Ag, ZnO, and TiO_2) into Transbond ™ XT adhesive primer helps to enhance the antibacterial properties of primer against the *Streptococcus mutans*.

REFERENCES

1. Artun J, Brobakken BO. Prevalence of carious white spots after orthodontic treatment with multi bonded appliances. Eur J Orthod 1986; 8(4): 229-34.
2. Al-Musallam TA, Evans CA, Drummond JL, Matasa C, Wu CD. Antimicrobial properties of an orthodontic adhesive combined with cetylpyridinium chloride. Am J Orthod Dentofacial Orthop 2006; 129(2): 245-51.
3. Xia Y, Zhang F, Xie H, Gu N. Nanoparticle-reinforced resin-based dental composites. J Dent 2008; 36(6): 450-5.
4. Ahn SJ, Lee SJ, Kook JK, Lim BS. Experimental antimicrobial orthodontic adhesives using nanofillers and silver nanoparticles. Dent Mater 2009; 25(2): 206-13.
5. Lanone S, Rogerieux F, Geys J, Dupont A, Maillot-Marechal E, Boczkowski J, Lacroix G, Hoet P. Comparative toxicity of 24 manufactured nanoparticles in human alveolar epithelial and macrophage cell lines. Part Fibre Toxicol 2009; 6: 14.
6. Arai T, Ueda T, Sugiyama T, Sakurai K. Inhibiting microbial adhesion to denture base acrylic resin by titanium dioxide coating. J Oral Rehabil 2009; 36(12): 902-8.
7. Poosti M, Ramazanzadeh BA, Zebarjad M, Javadzadeh P, Naderinasab M, Shakeri MT. Shear bond strength and antibacterial effects of orthodontic composite containing TiO2 nanoparticles. European J Orthodont 2013; 35(5): 676-9.
8. Sodagar A, Sadegh M, Akhoundi MSA, Bahador A, Jalali YF, Behzadi Z, Elhaminejad F, Mirhashemi AH. Effect of TiO_2 nanoparticles incorporation on antibacterial properties and shear bond strength of dental composite used in Orthodontics. Dent Press J Orthodont 2017; 22(5): 67-74.
9. Hojati ST, Alaghemand H, Hamze F, Ahmadian BF, Rajab-Nia R, Rezvani MB, Kaviani M, Atai M. Antibacterial, physical and mechanical properties of flowable resin composites containing zinc oxide nanoparticles. Dent Mat 2013; 29(5): 495-505.
10. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramírez JT, Yacaman MJ. The bactericidal effect of silver nanoparticles. Nanotechnology 2005; 16(10): 2346-53.
11. Hernández-Sierra JF, Ruiz F, Pena DC, Martínez-Gutiérrez F, Martínez AE, Guillén AJ, 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.
12. Kasraei S, Sami L, Hendi S, AliKhani M-Y, Rezaei-Soufi L, Khamverd Z. Antibacterial properties of composite resins incorporating silver and zinc oxide nanoparticles on Streptococcus *mutans* and *Lactobacillus.* Restorative Dent Endod 2014; 39(2): 109-114.
13. Blöcher S, Frankenberger R, Hellak A, Schauseil M, Roggendorf MJ, Korbmacher-Steiner HM. Effect on enamel shear bond strength of adding microsilver and nanosilver particles to the primer of an orthodontic adhesive. Bio Med Central Oral Health 2015; 15: 42.
14. 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 Orthod Sci 2016; 5(4): 127-131.
15. Al-Nafori1 MK, Mohamed G, Wael M. The Effect of Incorporating Gold and Silver Nanoparticles in Orthodontic Adhesive System on Bond Strength of Orthodontic Bracket. EC Dent Sci 2017; 11(4): 119-131.
16. Sari MN, Rahmani N, Kashani MA, Amirabadi GE, Sari AA, Seyedtabaii E. Effect of incorporation of nano-Hydroxyapatite and Nano-Zinc Oxide in resin modified glass ionomer cement on metal bracket debonding. Islamic Dent Associ Iran 2015; 27(2): 70-76.
17. Saffarpour M, Rahmani M, Tahriri M, Amir Peymani A. Antimicrobial and bond strength properties of a dental adhesive containing zinc oxide nanoparticles. Braz J Oral Sci 2016; 15(1): 66-9.
18. Heravi F, Moazzami SM, Ghaffari N, Jalayer J, Bozorgnia Y. Evaluation of shear bond strength of orthodontic brackets using trans-illumination technique with different curing profiles of LED light-curing unit in posterior teeth. Progress in Orthodontics 2013; 14(49): 1-5.
19. Jatania A, Shivalinga BM. An in vitro study to evaluate the effects of addition of zinc oxide to an orthodontic bonding agent. Eur J Dent. Jan-Mar 2014; 8(1): 112-117.
20. Gold OG, Jordan HV, Van Haute J. A selective medium for streptococcus mutans. Archs oral Biol 1973; 18(11): 1357-64.
21. Wade WG, Aldred MJ, Walker DM. An improved medium for isolation of *Streptococcus mutans*. J Med Microbiol 1986; 22(4): 319-23.
22. Lennette HE, Balows A, Shadomy J. Manual of clinical microbiology,1985.
23. Bridson EY. Oxoid manual 6th ed, Unipath Ltd., Wade Road, Basingstocke, UK, 1990.
24. Edwardsson S, Thylstrup A, Fejerskov O. Textbook of cariology. Munksgaard, Copenhagen 3rd ed., 1986.
25. Emilson CG. Prevelence of *Streptococcus mutans* with different colonial morphologies in human plaque and saliva. Eur J Oral Sci 1983; 91(1): 26-32.
26. Koneman EW, Winn WC, Allen SD, Allen S, Janda WM, Schreckenberger PC, Procop GW, Woods GL. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. Lippincott Williams & Wilkins, 2006.
27. William F, Vincent D. An Overview of the Genus *Streptococcus*, 2005.
28. Finegold SM, Baron EJ. Methods for Identification of Etiologic Agents of Infections Disease. In: Bailey and Scott's Diagnostic Microbiology.7th ed. St. Louis: The CV Mosby CO., 1986.
29. Joanne MW, Linda MS, Christopher JW. Prescott/ Harley/ Klein's Microbiology. 7th edition. McGraw-Hill. Higher Education. Elsiever, Washington, 2008.
30. Lee YH, Wong DT. Saliva: An emerging bio-fluid for early detection of diseases. Am J Dent 2009; 22(4): 241-8.
31. Moller AJR. Microbiological examination of root canals and periapical tissues of human teeth. Odontol Tidskr 1966; 74: 1-38.
32. Powell GL, Runnells RD, Saxon BA, Whisenant BK. The presence and identification of organisms transmitted to dental laboratories. J Prosthet Dent 1990; 64(2): 235-7.

33. Hussian AM, Jassim RK. Effect of sodium fluoride addition as a disinfectant on some properties of alginate impression material. J Bagh College Dent 2015; 27(1):70-6.
34. Issa MI, Abdul-Fatah N. Evaluating the effect of silver nanoparticles incorporation on antifungal activity and some properties of soft denture lining material. J Bagh College Dent 2014; 27(2): 17-23.
35. Baron E, Peteson L, Fingold S. Methods for testing antimicrobial effectiveness. In: Bailey and Scotts Diagnostic Microbiology. 9th ed. CV Mosby Co, St. Louis, 1994.
36. Harley JP, Prescott LM. Laboratory Exercises in Microbiology, 5th ed. New York, NY, 2002.
37. Casemiro LA, Martins CHG, Panzeri FDC, Souza P, Panzeri H, Ito IY. Bacterial, fungal and yeast contamination in six brands of irreversible hydrocolloid impression materials. Braz Oral Res 2007; 21(2): 106-11.
38. Shakir TA, Abass SM. The Effect of Magnesium Oxide (MgO) Nano Fillers on the Antibacterial Activity and Some Properties of Heat Cured Acrylic Resin. Int J Sci Res 2016; 7(3): 2319-7064.
39. Nolte WA. Oral microbiology: with basic microbiology and immunology, 1982.
40. Aryal S. Catalase Test- Principle, Uses, Procedure, Result Interpretation with Precautions, 2018.
41. Köhler B, Andréen I, Jonsson B. The earlier the colonization by *mutans streptococci*, the higher the caries prevalence at 4 years of age. Oral Microbiol Immunol 1988; 3(1): 14-7.
42. Hamilton IR, Buckley ND. Adaptation by *Streptococcus mutans* to acid tolerance. Oral Microbiol Immunol 1991; 6(2): 65-71.
43. Marsh PD. Microbial ecology of dental plaque and its significance in health and disease. Adv Dent Res 1994; 8(2): 263-271.
44. Imazato S. Antibacterial properties of resin composites and dentin bonding systems. Dent Mater 2003; 19(6): 449-57.
45. Kumar R, Münstedt H. Silver ion release from antimicrobial polyamide/silver composites. Biomaterials 2005; 26(14): 2081-8.
46. Aydin Sevinç B, Hanley L. Antibacterial activity of dental composites containing zinc oxide nanoparticles. J Biomed Mater Res B Appl Biomater 2010; 94B(1): 22-31.
47. Guggenheim B, Giertsen E, Schupbach P, Shapiro S. Validation of an in vitro biofilm model of supragingival plaque. J Dent Res 2001; 80(1): 363-70.
48. Besinis A, De Peralta T, Handy RD. The antibacterial effects of silver, titanium dioxide and silica dioxide nanoparticles compared to the dental disinfectant chlorhexidine on *Streptococcus mutans* using a suite of bioassays. Nanotoxicology, 2014; 8(1): 1-16.
49. Ahrari F, Eslami N, Rajabi O, Ghazvini K, Barati S. The antimicrobial sensitivity of *Streptococcus mutans* and *Streptococcus sangius* to colloidal solutions of different nanoparticles applied as mouthwashes. Dent Res J 2015; 12(1): 44-9.

الخلاصة

واحده من اهم التعقيدات في علاج تقويم الاسنان الثابت هو تكوين البقع البيضاء، وهي بدايه الافه التسوسيه. ان اضافه عوامل مضاده للجراثيم الى ماده لصق تقويم الاسنان قد تكون الحل الحكيم لمنع تكوين البقع البيضاء. الاهداف من هذه الدراسه هي تقييم الخصائص المضاده لبكتيريا المكورات العقديه الميوتانز لـ (Transbond XT primer) نظام لصق تقويم الاسنان بعد اضافه ثلاث انواع مختلفه من جزيئات النانو(الفضه، اوكسيد الزنك، وثنائي اوكسيد التيتانيوم).

مجموعه من (40) قرص تم تحضيره، 10 اقراص مصنوعه من Transbond XT primer فقط و30 قرص مصنوعه من (الفضه ، اوكسيد الزنك، وثنائي اوكسيد التيتانيوم النانوية) بعد خلطها مع Transbond XT primer تم اختبار الخصائص المضاده للبكتريا عن طريق تحديد اعداد المستعمرات القابله للحياه للمكورات العقديه الميوتانز.

مقارنه التاثير المضاد للبكتريا بين المجموعه المسيطرعليها وباقي المجموعات تظهر فروقات كبيره بين المجاميع الاربعه حيث ان مجموعه ثنائي اوكسيد التيتانيوم النانوي تملك اقل قيمه لعدد المستعمرات وبعدها مجموعه اوكسيد الزنك ، وثم الفضه النانويه في حين المجموعه المسيطرعليها تملك اعلى قيمه لعدد المستعمرات.

في الختام، اضافه (الفضه، اوكسيد الزنك، او ثنائي اوكسيد التيتانيوم) النانوي الى primer في نظام اللصق في تقويم الاسنان سيعزز تاثيره المضاد للبكتريا وهذا يعتمد على حجم جزيئات النانو(حجم جزء النانو الاصغر يتملك النشاط المضاد للبكتريا الاعلى).