Evaluation of the Shear Bond Strength of Orthodontic Adhesive System Containing Antimicrobial Silver Nano Particles on Bonding of Metal Brackets to Enamel

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Abstract: *Objective:* The aim of this study was to evaluate the shear bond strength of orthodontic adhesive system containing antimicrobial Silver nanoparticles on bonding of metal brackets to enamel. *Material and Methods:* Forty freshly extracted human premolar teeth were embedded into PVC with chemically-cured acrylic resin. The labial surfaces were flattened and received prophylaxis with pumice and water. The teeth were divided into two groups according to the addition of silver nanoparticles to the adhesive system. Group 1: the adhesive system containing silver nanoparticles. Group 2: adhesive system only. The bonding was performed according to the manufacturers' recommendations. The least concentration of silver nanoparticles that has the highest antimicrobial activity was 0.5 µm. Using a graduated pipette 0.5µm silver nanoparticles in ethanol solution was added to every 1mm of the adhesive system (relay Bond). After acid etching, one coat of the bonding agent was applied and the brackets were bonded to etched enamel. The bonded test specimens were stored in distilled water at 37^0 c for 24 hours. The bonds were stressed to failure in an Instron machine at a crosshead speed of 0.02 inch per minute. The shear bond strengths were measured and recorded in megapascals (MPa). Statistical analysis was done. *Results:* The bond strength of the adhesive system containing no silver nanoparticles was statistically higher than that one containing. *Conclusion:* addition of silver nanoparticles to the bonding system affects the shear bond strength of the orthodontic brackets to enamel.

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Key words: Nonosilver, shear bond strength, Metal brackets

1.Introduction

Streptococcus mutans are considered the main cause for demineralizing enamel surfaces as well as increasing the surface roughness of composite resins[1]. As fixed orthodontic appliances present a difficulty for proper oral hygiene maintenance; they result in accumulation of bacterial films and plaque around the appliance with subsequent decalcification[2] mainly at the adhesive/enamel interface[3,4].

Many antimicrobial agents approved for intraoral use have proven to prevent demineralization when incorporated in orthodontic adhesives[5,6]. Some of which are Fluoride and chlorhexidine[7, 8], however they are only initially strong[2] and their effect doesn't last for long. In addition, manipulating commercial composite adhesives by adding antimicrobial agents has proven to have a set back on their mechanical properties[9, 10] as they cause a higher bond failure rate[10].

Recently it has been shown that silver ions are potent anti-microbial agents[11]. When antibacterial composites containing silver nanoparticles were prepared, an inhibition of both gram negative and gram positive bacterial growthoccurred[12]. They also have proven to be effective against streptococcus mutans and lactobacillus[13].

In orthodontics this characteristic is of particular interest since the plaque accumulation trap offered by orthodontic brackets invites white spot lesions and cavitation[4].

Accordingly, development of clinically acceptable orthodontic adhesives with additional anti-microbial features could be undertaken only if their mechanical properties have also been considered. Studies on the shear bond strength of brackets after incorporating silver nanoparticles within the adhesives were conducted to evaluate the feasibility of making use of its antibacterial properties, so it may seem valuable to assess adding silver nanoparticles to the adhesive system and evaluate their effect on the shear bond strength of orthodontic brackets to enamel.

2. Material and Methods

2.1. Material

The materials used in this study were0,56mm Roth brackets, 0.22 slot (Rx, 3M Gemini metal brackets), non fluoride Rely-a-bond no mix adhesive(Reliance Orthodontic Products, Itasca, III), and 1% Silver Nanoparticles (Sigma Aldrich ,St. Louis, MO).

2.2. Methods

Preparation and characterization of Silver Nanoparticles (AgNPs)

Chemical reduction reaction was used to prepare the silver nanoparticles. Silver nitrate ($AgNO_3$) was used as the silver precursor. 10 ml of 1% ethanolic solution of polyvinyl pyrrolidone (PVP, Sigma Aldrich St. Louis, MO), with average molecular weight = 22,000 and 0.2 ml of 0.1 m Silver Nitrate(Cary 5000 UV- Vis -NIR Spectrophotometer, GMI, USA) powder (AgNO3) were added to 25ml distilled water in a closed test tube. The tube was then placed in an oven; using microwave irradiation; that was operated at the 100% power of 1000W and frequency 2450MHz for 5 seconds. Immediate color change from colorless solution ***(fig. 1A)***,into characteristic pale yellow color***(fig.1B)***; indicating the formation of silver nanoparticles[14]**.**This solution was ultrasonically stirred for 1 hour, and then the obtained resultant solution concentration was 107µg/ml and finally ready for further characterization.

Fig. (1A): The solution inside the oven before heating (clear)

Fig. (1B): the solution oven after heating (yellow)

Characterization of Silver Nanoparticles

Two different methods were used for characterization of Silver nanoparticles: ***Firstly*** the produced silver nanoparticles were characterized using Ultraviolet–visible absorption (UV-Vis, Cary 5000 UV- Vis -NIR Spectrophotometer, GMI, USA) spectroscopy to determine the characteristic optical properties of Silver nanoparticles[15] ***(fig.2).***

Secondly Transmission electron microscope (TEM, JEOL JEM-2011 (JEOL Ltd, Japan)) was used to determine the size and shape of silver nanoparticle. Samples were prepared for TEM analysis by placing a drop of the solution on a carbon coated copper grid and drying in air. Images were recorded using a Gatan DualVision 600t CCD camera attached to the microscope and were then analyzed using Gatan Digital Micrograph Version 3.11.1. The TEM was calibrated for diffraction and imaging mode using standard samples[16].

Fig. (2): UV-Vis-NIR Spectrophotometer

The minimum concentration that provided the most effective antibacterial action (0.5µg/ml) according to Greulich *et al.*[17], and Shahrokh *et al.*[18], was prepared to be the concentration added to the adhesive. Using a graduated pipette 0.5µm silver

nanoparticles in ethanol solution was added to every 1mm of the adhesive system.

Specimen preparation

Forty freshly extracted human premolar teeth were used in the study; they were embedded in self-polymerizing acrylic. The labial surfaces received prophylaxis with pumice and water. The teeth were divided into two groups according to the addition of silver nanoparticles to the primer of the adhesive system(Rely-a-Bond). Group 1: the adhesive system containing silver nanoparticles. Group 2: the adhesive system without addition of silver nanoparticles. The labial enamel surface was etched for 30 s with a 37% phosphoric acid gel, rinsed with a water syringe for 60 s and dried. The primer was applied to both the tooth surface and the bracket base. The adhesive paste was then applied to the bracket base and the brackets were placed in the center of the labial surface of the tooth. The brackets were seated in place using hand pressure and the excess adhesive was removed with the aid of a hand scaler. A halogen lamp – light curing device (3M Unitek, Monrovia, CA) was used for curing the adhesive10 s for each side of the bracket (a total of 40 sfor each specimen).

Shear bond strength procedure

Shear bond strength testing was performed with a universal testing machine ((Model LRX-plus; Lloyd Instruments Ltd., Fareham, UK),the shearing force was applied with the aid of a chiselshape blade having a 0.6 mm thick edge. An occluso-gingival force with a cross-head speed of 0.5 mm/min was applied to the specimens until failure occurs and the maximum loading force was recorded in Newtons. These figures were divided by a nominal bracket base surface area of 12.62 mm2, in order to convert the values into MegaPascals (MPa). Finally, the specimens were examined under a stereo microscope with a 10X magnification in order to determine the adhesive remnant index (ARI) as introduced by Artun and Bergland[19].

Failure Mode

After debonding, all teeth and brackets in the test groups were viewed using a USB digital-microscope (Scope Capture Digital Microscope, Guangdong, China), magnification X65, and the images were captured and transferred to a IBM personal computer equipped with the Image-tool software (Image J 1.43U, National Institute of Health, USA) to determine the bracket failure interface.

Any adhesive remaining after debonding was assessed and scored according to the modified adhesive remnant index (ARI; Olsen *et al*[20])

The scoring criteria of the index are as follows:

Score 1 = all of the composite, with an impression of the bracket base remains on the tooth;

Score 2 = more than 90per cent of the composite remains on the tooth; ***Score 3*** = more than 10 per cent but less than 90 per cent of the composite remains on the tooth;

Score 4 = less than 10 per cent of composite remains on the tooth; and ***Score 5*** = no composite remains on the tooth.

Data analysis was performed with the aid of version16 of the SPSS software (SPSS, IL).Descriptive statistics and one-way analysis of variance(ANOVA) were done.

3. Results

3.1. Results of Characterization of Silver Nanoparticles

Nanosilver optical properties

The absorption rate of the resultant solution was with a maximum peak of 422 nm indicating presence of spherical or roughly spherical silver nanoparticles (Figure 3).

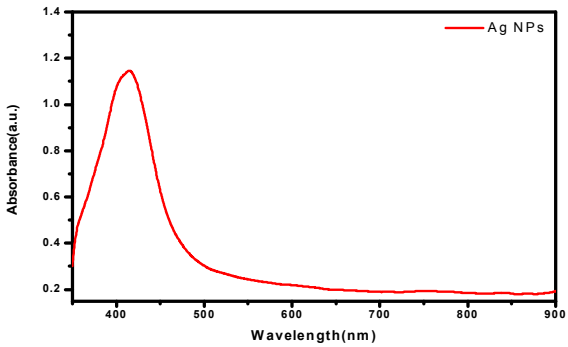


Figure (3): the absorption rate curve of silver nanoparticles solution

3.2. Results of Size, shape and particles' distribution

The resultant TEM images recorded and analysed on the computer at 50 and 100 nm scales showed that the resultant spherical particles of 4-10 nm in diameter. The particles were observed to be highly monodispersed and uniformly distributed (Figures 4, 5).

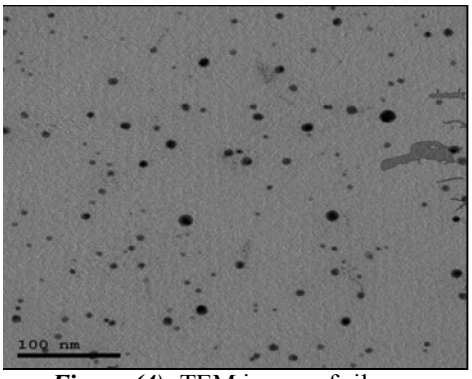


Figure (4): TEM image of silver nanoparticles (100 nm scale).

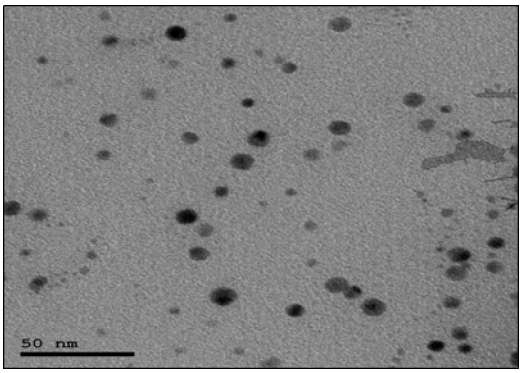


Figure (5): TEM image of silver nanoparticles (50 nm scale).

3.2.1. Shear Bond Strength

Table (1) demonstrated a descriptive study of the shear bond strength measurements between the different composite adhesive groups plotted as means and standard deviations.

There was a significant difference between the measurements of both groups, as (*P*-value < 0.05).

3.2.2. Failure Mode

The changes in measurements were scored into five different scores from 1-5 according to ARI score system which were

Score 1 = all of the composite, with an impression of the bracket base remains on the tooth;

Score 2 = more than 90per cent of the composite remains on the tooth

Score 3 = more than 10 per cent but less than 90 per cent of the composite remains on the tooth

Score 4 = less than 10 per cent of composite remains on the tooth

Score 5 = no composite remains on the tooth.

Samples were counted for each score with each type of adhesive material to calculate O.R (Odds Ratio) for estimation of the R.R (relative risk) of using both different types of adhesive material. This was performed at 95% CI (Confident Interval).

Risk values were calculated between both groups which revealed insignificant difference (O.R<1) between both types (Low Risk) (Table 3, Figure 6-7).

Table (1): Shear bond strength measurements

		Mean± SD	df	F	*P*-value
Shear bond strength	Conventional	3.55 ± 1.77	38	27.787	.001**
	Nano silver containing adhesive	2.16 ± 0.43			

*df; Degree of Freedom, F; variable, P; Probability Level, **significant difference*

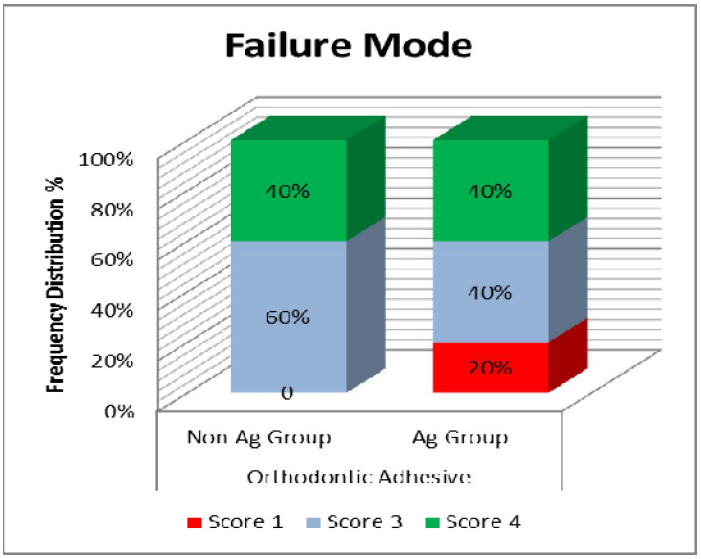
Table (2): Frequency distribution (%) of the adhesive remnant index (ARI) scores for both groups:

	ARI score	
	No Ag Group	*Ag Group*
Score 1	0	4(20%)
Score 2	0	0
Score 3	12(60%)	8(40%)
Score 4	8(40%)	8(40%)
Score 5	0	0

Table (3): Relative risk of both adhesive types

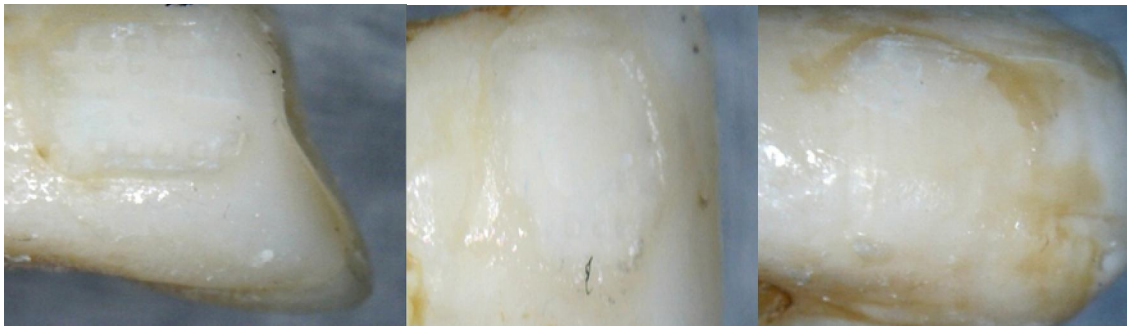
Frequency	95% Cl	O.R	Sig.
Bad Outcome	0.23-1.33	0.5	No
Good Outcome			

Cl; Confident interval, O.R; Odds ratio, Sig; Significance



Green; Good Outcome, Red; Bad Outcome

Figure (6): Bar chart resembling failure scoring of both adhesive materials



(A) **(B)** **(C)**

Fig.(7A) Microscopic image showing score 1 mode of failure
Fig. (7B) score 3 mode of failure
Fig. (7C) score 4 mode of failure

4. Discussion

Plaques adjacent to the restoration margins could result in secondary caries and compromise the restoration's longevity[21,22]. Therefore, there were great efforts to improve the longevity of composite restorations by incorporating bioactive agents to combat microbial destruction and recurrent caries while sustaining the load-bearing capability, such as zinc oxides nanoparticles, amorphous calcium phosphate nanoparticles, quaternary ammonium dimethacrylate and silver nanoparticles[23,24,25]. Silver (Ag) is known to have antibacterial, antifungal, and antiviral capabilities[26].

The purpose of this study was to evaluate the effect of incorporation of silver nanoparticles into adhesive used to bond orthodontic brackets on its shear bond strength and compare it without silver incorporation.

Silver nanoparticles was chosen in this study and incorporated into adhesive resin because it is a stable and durable material that cannot be dissoluted or degraded by body fluids. Silver and silver salts were used for decades as antimicrobial agents in curative and preventive health care. It was incorporated in drugs; as a delivery system, in order to achieve the site-specific action of the drug at the therapeutically optimal rate and minimizing side effects[27].

Nano-silver, a particle of Ag element, is a new class of material having remarkably different physio-chemical and biological characteristics such as increased optical, electromagnetic, catalytic properties and antimicrobial activity than bulk materials[27,29]. Reducing the particle size of materials is an efficient and reliable tool for improving their biocompatibility. Actually, nanotechnology helps in overcoming the limitations of size and can change the

outlook of the world regarding science. Moreover, the antimicrobial effect of silver nanoparticles against different micro-organisms was confirmed in previous studies as[18,29-36], Silver nanoparticles can attach to bacterial cell wall, thus decreasing its permeability and cellular respiration because of its decreased solubility in aqueous medium. The nanoparticles can also penetrate inside the cell causing damage by interacting with its phosphorus and sulphur containing compounds such as DNA and protein[33].

Chemical reduction or physical method can be two ways to prepare Silver nanoparticles. Preparation by conventional heating or microwave mediated synthesis as suggested by Kim et al[28]. Silver nanoparticles ethanol based solution was prepared in this study through reduction method using microwave. The improved kinetics of the reaction; due to rapid initial heating and generation of localized high-temperature zones at reaction site are considered advantages for this method[14].

Silver nanoparticles solution stabilized by polyvinyl pyrrolidone (PVP) has the highest antibacterial efficacy among different stabilizing agents[36].That's why it was necessary to use a stabilizing agent (PVP) to control the reduction rate of the silver ions and the aggregation process of the metal particles, after the interaction between the silver moieties and nitrogen atom of the PVP[14,17,28].

Ethanol based silver nanoparticles was used in the current research as ethanol evaporates after application within few seconds, leaving precipitated silver nanoparticles on the tested area.

It has been observed that size and shape of silver nanoparticles can affect their antibacterial performance. They have the largest antibacterial effects with the smallest particle sizes, with average diameters under 10 nm being most effective[29,37]. Consequently; characterization of silver nanoparticles was done to detect their specific size[28,29,31,33,36]. Spherical nanoparticles, with a total silver content above 12.5 µg reduced the number of colonies significantly, while a total of 50 to 100 µg of silver caused 100% inhibition of bacterial growth[31].

The concentration of nanosilver particles chosen in the current study was 0.5µg/ml.It was found that this concentration provided the best effective antibacterial action[17]

The incorporation of silver nanoparticles into resin can be done in several methods. Cheng *et al.*[21], Zhang *et al.*[22] and Melo *et al.*[25] dissolved silver 2-ethylhexanoate powder into 2-(tertbutylamino) ethyl methacrylate by stirring, and then was added to the resin. Kasraei and Azarsina in 2012[38], mixed silver nanoparticles continuously with the resin composite using a plastic spatula for 30 min in a dark room, but it's claimed that hand mixing could cause agglomeration[22]**.** Therefore, due to the small size of silver nanoparticles, mechanical mixing with adhesive resin by mixer was done in this study, allowing the silver nanoparticles to be homogenously distributed through the specimen[39], which may affect mechanical strength positively[40].

Shear bond Strength Results:

The results in this study showed low shear bond strength of both groups, this may be in coincidence with many investigations dealing with the orthodontic SBS of adhesives comparing the results of their studies with previously published articles and the current investigation, claiming that a value between 6–8 MPa is a clinically acceptable figure, adequate to withstand occasional masticatory loads and at the same time low enough to prevent damage to the enamel[41]**.**

Data from the shear bond strength results revealed statistical significant difference between the measurements of the two groups,the conventional adhesive and the adhesive with addition of Ag nanoparticles; where the group incorporating Ag nanoparticles exhibited lower shear bond strength.

5. Conclusions

It has been proposed that nanofillers can improve adhesion at the interface between the restorative material and the tooth structure through increasing mechanical strength of the adhesive layer and providing structural reinforcement. In fact, the nanofillers are stress absorbing and have the role of an elastic layer between dental composite and enamel. However, the amount of nanofiller and distribution of particles are the critical parameters which should be optimized in experiments. Studies showed that uniform distribution of highly separated nanoparticles into dental resins/composites could significantly improve the mechanical properties of the resins/composites[42].

This was clear in our study as the decrease in bond strength associated with the addition of nanoparticles could be due to an agglomeration of particles inside the primer, creating defect points and interfering with the curing process of the material and eventually it reduced the bond strength, in contrast to Akhavan *et al*[43] as they used Hydroxyapatite (HA) added with silver in their study. They claimed that incorporation of silver/HA nanoparticles containing 1% silver significantly increase the orthodontic shear bond strength of Transbond XT as an orthodontic adhesive. Moreover, they added that increasing the amount of silver to 5% reduces the bond strength.

References

1. Beyth N, Bahir R, Matalon S, Domb AJ, Weiss EI.: Streptococcus mutans biofilm changes

surface-topography of resin composites. Dent Mater 2008;24:732-736.
2. Gorton J, Featherstone JD.:*In vivo* inhibition of demineralization around orthodontic brackets. Am J OrthodDentofacialOrthop 2003; 123:10-14. sited from Miresmaeili A, Atai M, Mansouri K and Farhadian N: Effect of nanosilver incorporation on antibacterial properties and bracket bond strength of composite resin; Iranian Journal of Orthodontics, Vol. 7, 2012, 14-19.
3. Arhun N, Arman A, Cehreli SB, Arikan S, Karabulut E, Gulsahi K.:Microleakage beneath ceramic and metal brackets bonded with a conventional and an antibacterial adhesive system. Angle Orthod 2006; 76:1028-1034.
4. Lim BS, Lee SJ, Lee JW, AhnSJ.: Quantitative analysis of adhesion of cariogenic streptococci to orthodontic raw materials. Am J OrthodDentofacialOrthop 2008; 133:882-888.
5. Saito K, Hayakawa T, Kawabata R, Meguro D, Kasai K.:Antibacterial activity and shear bond strength of 4-methacryloxyethyl trimellitate anhydride/methyl methacrylate-tri-n-butyl borane resin containing an antibacterial agent. Angle Orthod 2007; 77: 532-536.
6. Ahn SJ, Lee SJ, Kook JK, Lim BS.:Experimental antimicrobial orthodontic adhesives using nanofillers and silver nanoparticles. Dent Mater 2009; 25:206-213.
7. Sudjalim TR, Woods MG, Manton DJ, Reynolds EC.:Prevention of demineralization around orthodontic brackets in vitro. Am J OrthodDentofacialOrthop 2007;131:705 e701-709.
8. Vorhies AB, Donly KJ, Staley RN, Wefel JS.:Enamel demineralization adjacent to orthodontic brackets bonded with hybrid glass ionomer cements: an in vitro study. Am J OrthodDentofacialOrthop 1998;114:668-674.
9. Jedrychowski JR, Caputo AA, Kerper S.: Antibacterial and mechanical properties of restorative materials combined with chlorhexidines. J Oral Rehabil 1983;10:373–81.
10. Bulut B, Türkün M, Türkün LS, Isiksal E.: Evaluation of the shear bond strength of 3 curing bracket bonding systems combined with an antibacterial adhesive. Am J OrthodDentofacialOrthop 2007; 132: 77–83.
11. Karnib M., Holail H., Olama Z., Kabbani A. and Hines M.:The Antibacterial Activity of Activated Carbon, Silver, Silver Impregnated Activated Carbon and Silica Sand Nanoparticles against Pathogenic E. coli BL21, Int. J. Curr. Microbiol. App. Sci (2013) 2(4): 20-30.
12. Melinte V., Buruiana T., Moraru I. D. and Buruiana E. C.: Silver-Polymer Composite Materials With Antibacterial Properties; Digest Journal of Nanomaterials and Biostructures Vol. 6, No 1, January-March 2011, p. 213 – 223.
13. kasraei S, Sami I., Hendi S., AliKhani M.Y, Rezaei-Soufi L. and Khamverdi Z.: Antibacterial properties of composite resinsincorporating silver and zincoxide nanoparticles on streptococcus mutans and lactobacillus; Restor Dent Endod.2014 May;39(2):109-114.
14. Angshuman P., Sunil S. and Surekha D.: Microwave-assisted synthesis of silver nanoparticles using ethanol as a reducing agent. Materials Chemistry and Physics, 2009; (114): 530- 32.
15. Gurunathan, S.: Rapid biological synthesis of silver nanoparticles and their enhanced antibacterial effects against Escherichia fergusonii and Streptococcus mutans. Arabian Journal of Chemistry,(2014), http://dx.doi.org/10.1016/j.arabjc.2014.11.014.
16. Corrêa J.M., Mori M., Sanches H. L., da Cruz A. D., Poiate Jr. E., and Poiate I. A.: Silver Nanoparticles in Dental Biomaterials; International Journal of Biomaterials, 2015, 1-9
17. Greulich C., Kittler S., Epple M., Muhr G. and Köller M.: Studies on the biocompatibility and the interaction of silver nanoparticles with human mesenchymal stem cells (hMSCs). *Langenbeck's Archives of Surgery,* 2009; 394(3):495-502.
18. Shahrokh S. and Emtiazi G.: Toxicity and Unusual Biological Behaviour of Nanosilver on Gram Positive and Negative Bacteria Assayed by Microtiter-Plate. European Journal of Biological Sciences, 2009; 1(3): 28- 31.
19. Årtun, J. and S. Bergland.:Clinical trials with crystal growth conditioning as an alternative to acid-etch enamel pretreatment. Am J Orthod1984. 85:333–340.
20. Olsen M., Bishara S., Damon P, Jakobsen J R: Evaluation of Scotchbond Multipurpose and maleic acid as alternative methods of bonding orthodontic brackets. American Journal of Orthodontics and DentofacialOrthopedics 1997;111: 498–501
21. Cheng L., Weir M. D., Xu H. H. K., Antonucci J. M., Lin N. J., Lin-Gibson S., Xu S. M. and Zhou X.: *Effect of amorphous calcium phosphate and silver nanocomposites on dental plaque microcosm biofilms.* BiomedicalMaterialsResearchPartB*:* Applied Biomaterials,2012; 100 (5): 1378- 86.
22. Zhang K., Melo M.A., Cheng L., Weir M.D., Bai Y. and Xu H.H.:*Effect of quaternary ammonium and silver nanoparticles-containing adhesive on dentin bond strength and dental plaque microcosm biofilms.* Dental Materials, 2012; 28: 842- 852.
23. Jones N., Ray B., Ranjit K.T. and Manna A.C.:*Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms.*Federation of European Microbiological Societies (FEMS) Microbiology Letters, 2008; 279(1): 71- 76.

24. Chaloupka K., M.Y. and Seifalian A.M.:*Nanosilver as a new generation of nanoproduct in biomedical applications.*Trends Biotechnology, 2010; 28(11):580-8.
25. Melo M.A., Mary Anne S., Cheng L., Zhang K., Weir M.D., Rodrigues L.K. and Xu. H.H.:*Novel dental adhesives containing nanoparticles of silver and amorphous calcium phosphate.*Dental Materials, 2013; (29): 199-10.
26. Monteiro D.R, Gorup L.F, Takamiya A.S, Ruvollo-Filho A.C, Camargo E.R and Barbosa D.B.: The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver. International Journal of Antimicrobial Agents, 2009; 34:103-110.
27. Mohanraj V.J. and Chen Y.: Nanoparticles – A Review. Tropical Journal of Pharmaceutical Research, 2006; 5: 561-73.
28. Kim J.K., Choi J.W. and Koo K.:Reduction of Silver Nitrate in Ethanol by Poly (N-vinylpyrrolidone). *Journal* of Industrial and Engineering Chemistry, 2007; (13): 566- 70.
29. Guzmán M.G., Dille J. and Godet S.: Synthesis of silver nanoparticles by chemical reduction method and their antibacterial activity. International Journal of Chemical and Biological Engineering, 2009; 2:3.
30. Sondi I. and Salopek B.: Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. Journal of Colloid and Interface Science, 2004; (275): 177-182.
31. Pal S., Tak Y. K. and Song J. M.: Does the Antibacterial Activity of Silver Nanoparticles Depend on the Shape of the Nanoparticle? A Study of the Gram-Negative Bacterium Escherichia coli. Applied and Environmental Microbiology, 2007; 73(6): 1712- 1720.
32. Diaz M., Barba F, Miranda M, Guiti F., Torrecillas R. and Moya J.: Synthesis and Antimicrobial Activity of a Silver-Hydroxyapatite Nanocomposite. Journal of Nanomaterials, 2009; 498-505.
33. Wei D., Sun W., Qian W., Yongzhong Y. and Xiaoyuan M.: The synthesis of chitosan-based silver nanoparticles and their antibacterial activity. Elsevier, 2009; 344: 2375-82.
34. Hiraishi N., Cynthia K.Y., Nigel M., King, Tagami J., and Tay F. R.: Antimicrobial Efficacy of 3.8% Silver Diamine Fluoride and Its Effect on Root Dentin. Journal of Endodontics, 2010; 36: 1026-1029.
35. Irwin P., Martin J., Nguyen L.H., He Y., Gehring A. and Chen C.Y.: Antimicrobial activity of spherical silver nanoparticles prepared using a biocompatible macromolecular capping agent: evidence for induction of a greatly prolonged bacterial lag phase. Journal of Nanobiotechnology, 2010; (34): 3155- 3158.
36. El- Kheshen A. and Gad El-Rab S.F.: Effect of reducing agents on size of silver nanoparticles and their antibacterial effect. Der PhramaChemica, 2012; 4(1): 53- 65.
37. Sintubin L., B. De Gusseme, P. Van Der Meeren, B.F.G. Pycke, W. Verstraete, and N. Boon: The antibacterial activity of biogenic silver and its mode of action. Applied Microbiology and Biotechnology, 2011; 91: 153–62.
38. Kasraei S. and Azarsina M.: Addition of silver nanoparticles reduces the wettability of methacrylate and silorane-based composites. Brazilian Oral Research, 2012; 26(6): 505- 10.
39. Sevnic B.A. and Hanley L.:Antibacterial Activity of Dental Composites Containing Zinc Oxide Nanoparticles. Journal of Biomedical Materials Research Part B: Applied Biomaterials,2010; 94(1): 22- 31.
40. Cheng Y.J, Zeiger D.N, Howarter J.A, Zhang X, Lin N.J. and Antonucci J.M.: In situ formation of silver nanoparticles in photocrosslinking polymers. Biomedical Materials Research Part B, 2011; 97:124- 31.
41. Reynolds IR.: A review of direct orthodontic bonding. Br J Orthod 1975; 2: 171–8.
42. Miresmaeili A, Atai M, Mansouri K andFarhadian N: Effect of nanosilver incorporation on antibacterial properties and bracket bond strength of composite resin; Iranian Journal of Orthodontics, Vol. 7, 2012, 14-19.
43. Akhavan A, Sodagar A, Motjahedzadeh F and Sodagar K: Investigating the effect of incorporating nanosilver/nanohydroxyapatite particles on the shear bond strength of orthodontic adhesives; ActaOdontologicaScandinavica, 2013; Early Online, 1–5.

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