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THE EFFICACY OF NANOSILVER COATED STAINLESS STEEL BRACKETS IN PREVENTION OF ENAMEL DEMINERALIZATION IN ORTHODONTIC PATIENTS

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ABSTRACT

Objective: the aim of this study was to evaluate the effect of nanosilver coated stainless steel brackets in prevention of enamel demineralization during orthodontic treatment. **Subjects and Methods:** A randomized clinical trial was conducted on 32 patients aged 13-16 years. All participants were scheduled to have maxillary and mandibular first premolars extraction as a part of orthodontic treatment. The patients were randomly divided into two equal groups: Group I in which 64 nanosilver coated stainless steel brackets were bonded in the upper and lower first premolars, and Group II in which conventional uncoated stainless steel brackets were bonded in the upper and lower first premolars. In both groups, 32 brackets of one side from upper and lower arches were deboned after one month, and the other side after 2 months. After debonding, the teeth were extracted, cleaned, and prepared for evaluation under scanning electron microscope to investigate enamel surface topography, and energy dispersive x-ray analysis that evaluate the mineral content of the teeth. **Results:** Calcium & phosphorus contents in the extracted premolars were stable in group I after one and two months, while group II showed significant decrease in calcium & phosphorus contents at the same periods. Scanning electron microscope examination revealed almost normal topographic feature of enamel surface in group I, while enamel surface in group II showed typical features of enamel demineralization. **Conclusion:** Silver nanoparticles coated stainless steel brackets can preserve the mineral content of the enamel surface, thus decreasing the likelihood of demineralization during treatment than uncoated brackets.

KEY WORDS: silver nanoparticles, enamel demineralization, scanning electron microscopy, energy dispersive x-ray spectroscopy

INTRODUCTION

Orthodontic treatment is an elective procedure to improve the patient's dentofacial appearance. The complex design of fixed appliances provides a platform that leads to increased plaque accumulation around orthodontic brackets(1). So, the risk to develop demineralization area adjacent to orthodontic brackets is a major barrier in achieving this goal.

The appearance of white spot lesion during orthodontic treatments may worsen the whole outcome even if all other treatment goals have been achieved (2). White spot lesions can become noticeable around orthodontic brackets within one month after placement of fixed appliance, while

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the formation of regular caries usually takes at least six months. They are commonly appearing on the cervical and the middle thirds of the buccal aspects of teeth around the brackets, especially in the gingival area [3]. Once white spot lesions develop during treatment, the integrity of the enamel surface is compromised, and it might require further restorative procedure after the removal of brackets. Therefore, preventing these lesions is important for comprehensive and successful outcome of orthodontic treatment [4].

Neither standard caries prevention measures, nor using different methods are capable of effectively preventing the demineralization process. Thus, using antimicrobial agents applied by nanotechnology, to suppress cariogenic bacteria and thereby inhibit the development of new caries lesions, seems to be a rational approach during orthodontic treatment [5].

Silver nanoparticles are one of the most attractive metallic nanomaterials owing to their wide range of applications with excellent performance. It exhibits remarkable antimicrobial activity, even at a low concentration with low cytotoxicity and immunological response [6].

In dentistry, nanosilver has been proven to be effective in vitro against cariogenic bacteria. Multiple studies had proposed their use in different preparations, showing good results in the treatment of early dental caries and controlling plaque-related biofilms [7].

Farhadian et al., [8] observed a higher reduction in Streptococcus mutans colony counts in patients received removable retainers containing silver nanoparticles with 40 nm in size and 500 ppm in concentration relative to the control group. In addition, Hernández-Gómora et al., [9] recorded efficient antibacterial properties of elastomeric modules impregnated with silver nanoparticles. Furthermore, Mirhashemi et al., [10] revealed the antimicrobial efficacy of composite resins containing nanosilver particles used in fixed orthodontic retainers.

Previous in vitro studies [11-14] evaluated the antibacterial effect of stainless-steel brackets, wire and bands coated with silver nanoparticles. The results suggested the potential of nanosilver to inhibit dental biofilm and in turn, decrease the incidence of demineralization in dental enamel. T7hey recommended carrying out further research in patients treated orthodontically.

The previously mentioned in-vitro results revealed excellent antimicrobial activity of silver nanoparticles. However, the number of studies confirming these positive in-vitro results, in the clinical situation, is very low. So, the present study was conducted to evaluate the effect of nanosilver coated stainless steel brackets in prevention of enamel demineralization in orthodontic patients.

MATERIALS AND METHODS

A randomized clinical trial was adopted in this study. The study design was approved by the Research, Ethics Committee of Faculty of Dentistry, Tanta University, Egypt (Code of Ethics #R-ORTH-2-19-1). The purpose of the study was explained to the patients' parents and informed consent was taken from them considering the guidelines on human research adopted by the Research Ethics Committee at Faculty of Dentistry, Tanta University. Scanning electron microscopic examination was done at the Faculty of Agriculture, Mansoura University.

The sample size calculation was performed using Epi-info software statistical package created by WHO on CDC (center of disease prevention and control) Atlanta, Georgia USA. Version 2002. The sample size was calculated using a confidence level of 95% Power of study 80%. The calculated sample size was estimated to be 16 patients, in each group.

All participants were selected from patients seeking orthodontic treatment at the Department of Orthodontics, Faculty of Dentistry, Tanta University. They were scheduled to have maxillary and mandibular first premolars extraction as a part of orthodontic treatment, with age ranging from 13-16 years. The enamel surface of the premolar teeth should be intact with no caries, hypoplastic areas, restorations, or cracks. None of the patients have been previously treated with fixed orthodontic appliances.

Coating procedure:

Silver nanoparticles were prepared using the biological method. All stainless steel brackets (mini diamond, Ormco Co., USA) were ultrasonically cleaned for five minutes to remove any impurities. Coating of the brackets was made via chemical bath deposition technique. The brackets were coated with uniform distribution of silver nanoparticles thin film with average size 25± nm and spherical shape, without any agglomerations and with a minimum lethal dose concentration (62.2 µg/ml).

The patients were randomly divided into two equal groups: group I (experimental group) 64 nanosilver coated stainless steel brackets were bonded in the upper and lower first premolars using 3M Transbond TM XT light cure adhesive bonding material, and group II (control group) in which conventional uncoated stainless steel brackets were bonded in the upper and lower first premolars. T-loops stainless steel 0.014-inch wire were engaged on brackets with elastomeric rings to simulate orthodontic procedures (Fig 1). In both groups, 32 brackets of one side from upper and lower were deboned after one month, and the other side after 2 months, to evaluate the clinical results at two intervals.

According to debonding schedule time of the study (one and two months), the patients were recalled for debonding brackets, and the teeth were extracted, cleaned from remnants of soft tissue. The roots were separated from the crown with a diamond disc under water spray just occlusal to the cement-enamel junction. After washing with distilled water, all crowns were stored in 0.1% thymol solution at 4^{o} C until required.

FIG (1) Intraoral images showing bonded stainless steel brackets and ligation of T-loop with elastic o-Ties.

X-ray energy dispersive spectroscopy (Oxford Instruments X-Max EDS system, Oxford. UK) was used to assess mineral content of the extracted premolar teeth (calcium and phosphorus ions). Scanning electron microscopy (SEM, JEOL, JSM-6510LV at 30 KV, Tokyo, Japan) was used to examine the buccal surface of the teeth around orthodontic brackets to identify the areas of enamel surface alterations.

Statistical analysis

The data were collected and analyzed using SPSS Version 21.0 (SPSS Inc, USA, III). Independent t-test was used for comparing the mineral content existed between the two groups.

RESULTS

Energy dispersive x-ray spectroscopy statistical analysis of the mean value by weight % of calcium and phosphorous contents in the study groups:

The mean values of calcium & phosphorus contents in the upper and lower first premolars after one and two months were stable in the experimental group, while in the control group there was a significant decrease in calcium & phosphorus mean values at the same periods. A decrease in calcium and phosphorus weight% in the upper first premolars was significantly greater than the lower first premolars throughout the study periods. (Table 1- 4).

Table (1) Comparison of the mean values of calcium weight% between the study groups after one month of bonding.

After one month	Calcium						t-test	
	Control (n=64)			**Experimental (n=64)**				
	Mean	**±**	**SD**	**Mean**	**±**	**SD**	**t**	**p-value**
Upper premolar	29.246	±	0.758	37.670	±	0.292	-42.550	**0.000***
Lower premolar	30.895	±	0.698	37.758	±	0.229	-37.156	**0.000***
t		-6.399			-1.059			
p-value		**0.000***			0.298			

SD= *Standard Deviation* Highly statistically significant difference p ≤ 0.001.*

Table (2): Comparison of mean values of calcium weight % between the study groups after two months of bonding.

After two months	Calcium						t-test	
	Control (n=64)			**Experimental (n=64)**				
	Mean	**±**	**SD**	**Mean**	**±**	**SD**	**t**	**p-value**
Upper premolars	26.363	±	0.548	37.805	±	0.218	-77.598	**0.000***
Lower premolars	28.911	±	0.669	37.835	±	0.244	-50.105	**0.000***
t		-11.776			-0.367			
p-value		**0.000***			0.716			

SD= *Standard Deviation * Highly statistically significant difference p ≤ 0.01.*

Table (3): Comparison of the mean values of phosphorus weight % between the study groups after one month of bonding.

After one month	**Phosphorus**						**t-test**	
	Control (n=64)			Experimental (n=64)				
	Mean	**±**	**SD**	**Mean**	**±**	**SD**	**t**	**p-value**
Upper premolar	13.793	±	0.567	19.590	±	0.230	-37.895	**0.000***
Lower premolar	14.817	±	0.778	19.658	±	0.190	-24.172	**0.000***
t		-4.252			-0.907			
p-value		**0.000***			0.372			

SD=*Standard Deviation *Highly statistically significant difference p ≤ 0.01*

Table (4): Comparison of mean values of phosphorus weight % between the study groups after two months of bonding.

After two months	Phosphorus						t-test	
	Control (n=64)			Experimental(n=64)				
	Mean	±	SD	Mean	±	SD	t	p-value
Upper premolar	11.433	±	0.704	19.706	±	0.295	-43.370	**0.000***
Lower premolar	13.410	±	0.307	19.721	±	0.251	-63.691	**0.000***
t		-10.302			-0.161			
p-value		**0.000***			0.873			

SD= *Standard Deviation *Highly statistically significant difference $p \leq 0.01$*.

Scanning electron microscopy evaluation:

Experimental group (Coated brackets):

The scanning electron microscope examination of enamel surface around coated brackets of maxillary and mandibular first premolars after one and two months of bonding revealed almost normal topographic feature of enamel surface with few scattered superficial depressions, some scratched and area of roughness. The stria of Ratizus was observed in some teeth without demarcation of perikymata overlapping with a little scratched that normally found in the enamel surface (Fig 2,3).

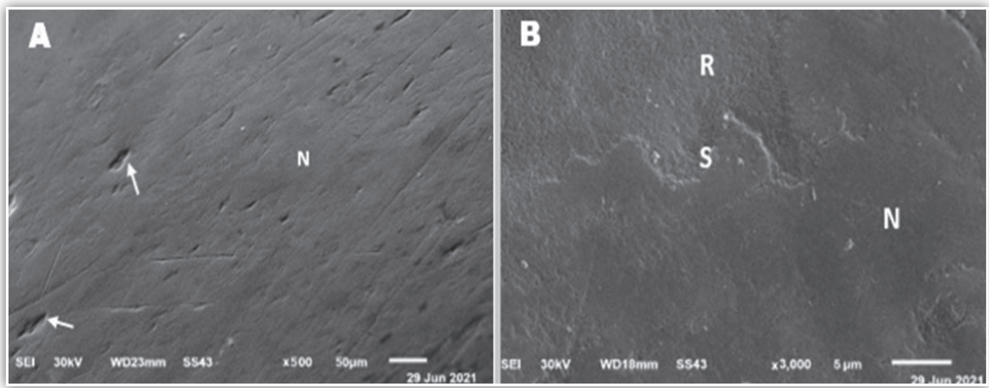

FIG (2) After 1 month (A) and 2 months (B) of bonding in the upper premolars in the experimental group, the scanning electron microscopy images show tinny areas of superficial scattered erosion **(White Arrows)** & normal line between the perikymata which represent the external manifestation of striae of Retzius **(S),** between normal enamel **(N)** and rough enamel surface **(R).**

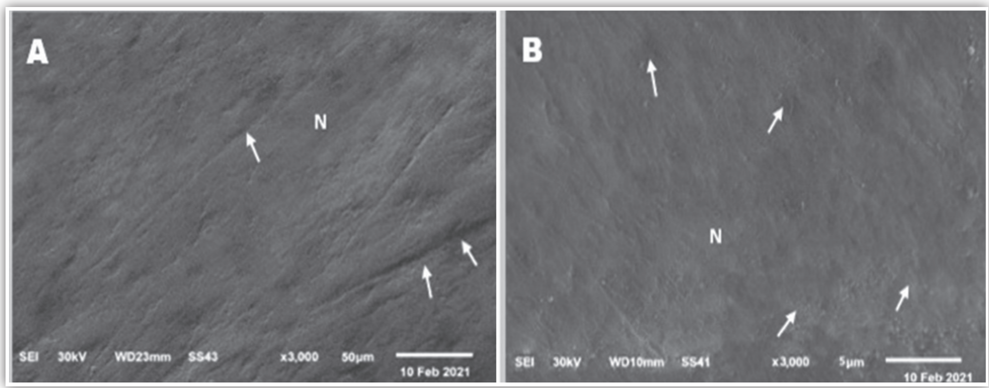

FIG (3) After 1 month (A) and 2 months (B) of bonding in the lower premolars in the experimental group, the scanning electron microscopy images show almost normal enamel surface **(N)** with few scratched superficial depressions & some roughness areas **(white Arrow)**.

Control group (Uncoated brackets):

After one month of bonding, scanning electron microscope examination of maxillary first premolars, at low magnification, the perikymata were pronounced due to the dissolution of the adjacent prism ends. At higher magnification, the prism cores showed more dissolution, donating more enamel destruction (Fig 4).

The lower first premolars enamel surface showed at low magnification, the presence of accentuating perikymata overlapping and area of focal holes depressions due to initial dissolution of the prism ends. A few focal holes were also seen scattered throughout the enamel surface with dissolution of Tomes' process ends (Fig 5).

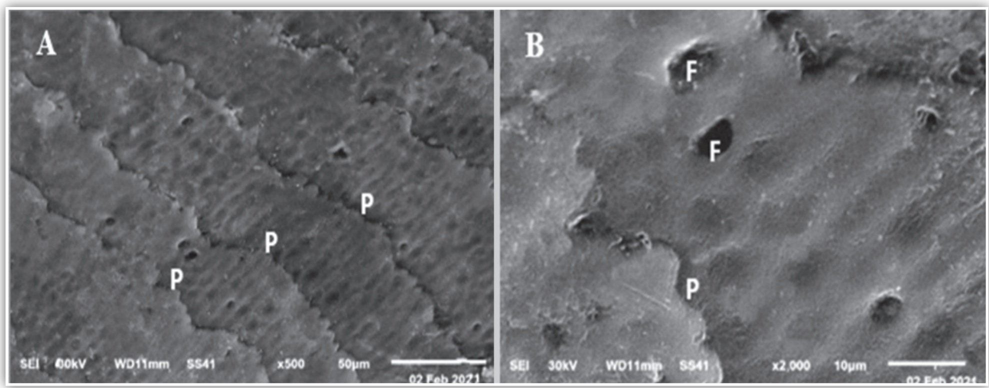

FIG (4) After 1 month of bonding in the upper premolars in the control group, the scanning electron microscopy images show accentuation of perikymata overlapping **(P),** with many focal holes **(F)**.

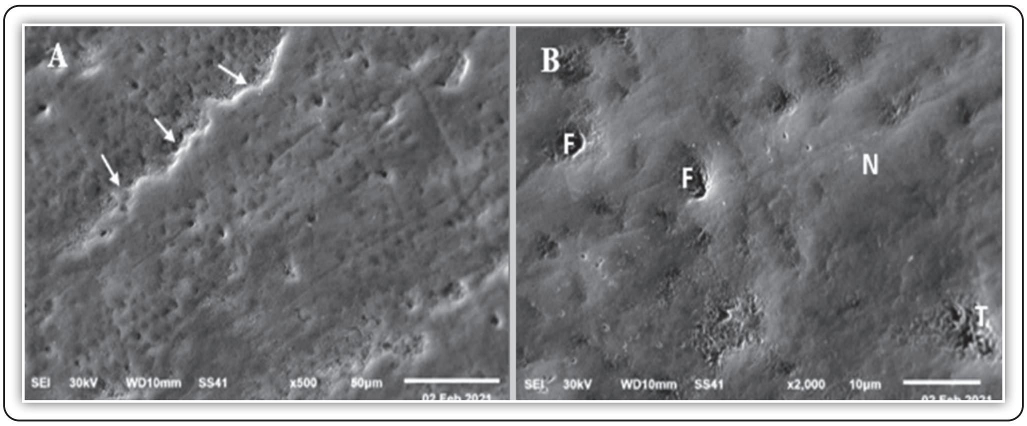

FIG (5) After 1 month of bonding in the lower premolars in the control group, the scanning electron microscopy images show accentuated perikymata overlapping **(White Arrow),** with scattered areas of focal holes **(F)** and extensive dissolution of Tomes' process ends **(T)** with area of normal enamel **(N).**

After two months, the enamel surface of the lower first premolars showed two main structural features, accentuation of the perikymata and deep focal micropits. The perikymata were flattened so that they had a distinct shelf like border, which was irregular in outline, and numerous deep focal holes spread throughout the surface. They had a characteristic funnel shape, being wider at the surface than deeper in the enamel (Fig 6).

In the upper first premolars, the direct dissolution become evidence at low magnification. The dissolution thus cause removal of the perikymata overlapping, whereby the underlying enamel were exposed. The dissolved prism ends gave the enamel surface an appearance of honeycomb appearance where the prism core was destroyed, whereas the interprismatic substance was less affected (Fig 7).

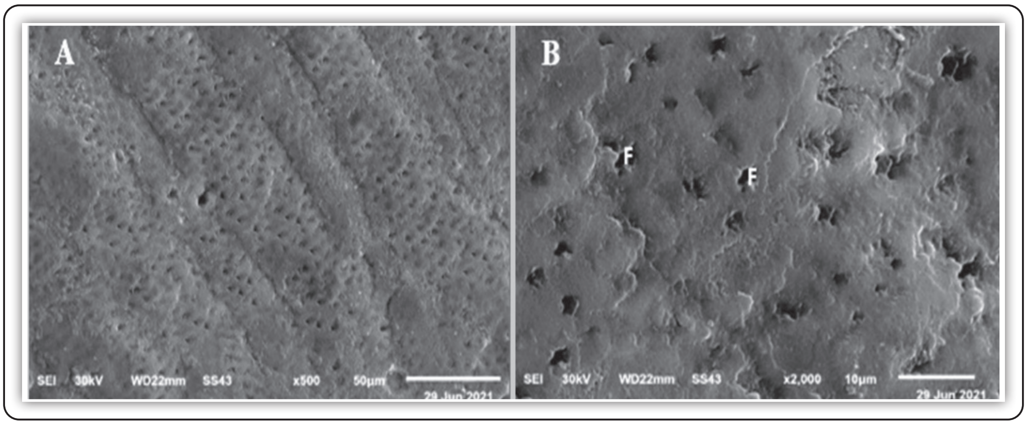

FIG (6) After 2 months of bonding in the lower premolars in the control group, the scanning electron microscopy images show more accentuation of the perikymata and development of isolated holes that spread throughout the surface **(F).**

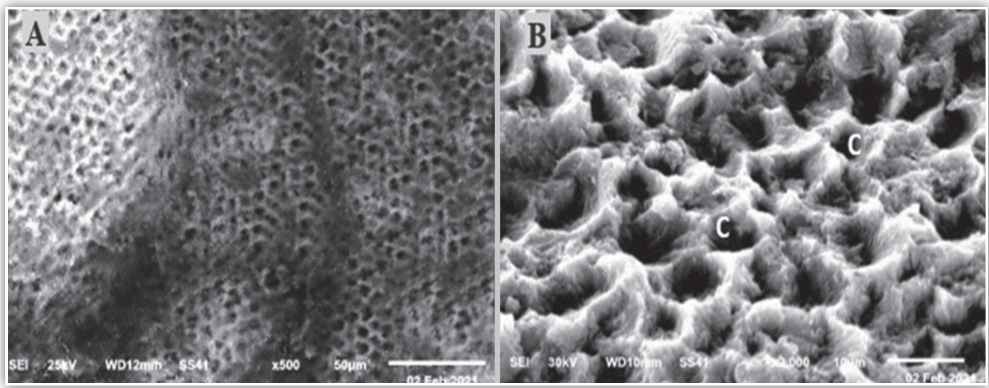

FIG (7) After 2 months of bonding in the upper premolars in the control group, the scanning electron microscopy images show complete erosion in the surface of perikymata exposed the underlying rods. The prism core was destroyed **(C)** whereas the interprismatic space was less affected with typical network of honeycomb appearance.

DISCUSSION

Despite the advances to improve the orthodontic practice, white spot lesions are among the most undesired side effects of fixed orthodontic treatment. Thus, it seems reasonable to search for a strategy to prevent these lesions rather than restoring them. The present study was conducted as a randomized controlled trial, to evaluate the ability of nanosilver coated stainless steel brackets in prevention of enamel demineralization in orthodontic patients.

Two different groups of patients were used in this study instead of split mouth technique, as silver nanoparticles can release silver ions when coming in contact with saliva in the oral cavity. This will affect the accuracy of the obtained results [15]. The upper and lower first premolars were used because the pattern of biofilm accumulation and white spot lesions distribution in the maxillary arch was different than the mandible. White spot lesions occur 2.5 times more frequently in the maxillary arch than the mandibular arch [16]. Although, significant and measurable mineral loss around fixed appliances was reported early after one month of the beginning of orthodontic treatment, emphasizing loss of minerals may go undetected by the clinician [17]. So, in the present study, two months' measurement was taken to evaluate the alteration of enamel surface over a long period of time. Energy dispersive x-ray analysis was used to assess the amount of mineral loss from the enamel surface. It is considered the gold standard for the evaluation of mineral loss or gain in experimentally induced initial caries lesions quantitatively [18]. While scanning electron microscope was used in to identify prismatic structural changes that occur at the enamel surface at the area of interest [19].

The obtained in vivo results from the present study cannot be compared with the in vitro results of other experimental studies reported so far. In the current study, energy dispersive x-ray analysis showed a significant decrease in calcium and phosphorus weight% of enamel content around orthodontic brackets in the control group. A significant reduction in calcium and phosphorus weight% in the upper first premolars was greater throughout the study periods compared to the lower first premolars. This can be attributed to increased remineralization capacity of saliva, that acts as a source of calcium and phosphate ions, in the lower arch than the upper one because the anatomic situation of the salivary glands and gravity induce lower teeth more susceptible to contact with saliva than the upper teeth [20].

In the experimental group, there was no significant difference change between calcium and phosphorus after one and two months in the upper and lower premolars throughout the study periods. These findings proved that silver nanoparticles have the ability to restrict the loss of mineral contents of the teeth. Silver nanoparticles can effectively reduce the incidence and prevalence of white spot lesions due to their antibacterial effect [(14)].

Concerning scanning electron microscope results, the enamel topographical features of the experimental group showed almost normal enamel surface with minimal superficial changes. This may be attributed to the ability of silver nanoparticles to decrease the dissolution of enamel structures by preventing acidogenic bacterial adhesion as the literature reports [(21,22)] that, the positive charges of the metal ions repel the negative charges of the bacterial membrane.

As regarding to the control group, the enamel surface showed typical features of enamel demineralization, surface layer has small depressions, which is a result of the dissolution of enamel prism ends, and the accentuated perikymata were due to the dissolution of the adjacent prism ends. A more extensive dissolution of Tomes' process ends was a common observation. After two months of bonding, a more advanced stage of dissolution of enamel structures was detected. So, it is reasonable to consider the adherence of cariogenic bacteria increase with time, producing lactic acid that drops pH and dissolves calcium & phosphors content from the enamel surface [(23,24)].

CONCLUSION

According to the data reported in the present study, it was concluded that:

1. The use of stainless steel brackets coated with silver nanoparticles demonstrate an excellent effect in prevention of enamel demineralization during treatment of orthodontic patient.
2. Nanosilver coated stainless steel brackets can preserve the mineral content of the enamel surface for a period of one and two months.

RECOMMENDATION

Other studies are needed to evaluate the effect of coating stainless steel brackets with silver nanoparticles on shear bond strength of orthodontic brackets.

REFERENCES

1. Cerroni S, Pasquantonio G, Condò R, Cerroni L. Orthodontic fixed appliance and periodontal status: an updated systematic review. The Open Dentistry Journal. 2018; 12:614-22.
2. Srivastava K, Tikku T, Khanna R, and Sachan K. Risk factors and management of white spot lesions in orthodontics. Journal of Orthodontic Science. 2013; 2: 43-9.
3. Kachuie M, Khoroushi M. Prevention and treatment of white spot lesions in orthodontic patients. Contemporary Clinical Dentistry. 2017; 8:11-9.
4. Amaechi B, McGarrell B, Luong M, Okoye L, Gakunga P. Prevention of white spot lesions around orthodontic brackets using organoselenium-containing antimicrobial enamel surface sealant. Heliyon. 2021;7: e06490-495.
5. Song W, Ge S. Application of antimicrobial nanoparticles in dentistry. Molecules. 2019; 24:1033-048.
6. Yin I, Zhang J, Zhao I, Mei M, Li Q, Chu C. The antibacterial mechanism of silver nanoparticles and its application in dentistry. International Journal of Nanomedicine. 2020; 15: 2555-562
7. Tan P, Li H, Wang J, Gopinath S. Silver nanoparticle in biosensor and bioimaging: Clinical perspectives. Biotechnology and Applied Biochemistry. 2021; 68: 1236-242.
8. Farhadian N, Usefi-Mashoof R, Khanizadeh S, Ghaderi E, Farhadian M, Miresmaeili A. Streptococcus mutans counts in patients wearing removable retainers with silver nanoparticles vs those wearing conventional retainers: A randomized clinical trial. American Journal of Orthodontic and Dentofacial Orthopedic. 2016; 149:155-60.
9. Hernández-Gómora A, Lara-Carrillo E, Robles-Navarro J, Scougall-Vilchis R, Hernández-López S, Medina-Solís C, et al. Biosynthesis of silver nanoparticles on orthodontic elastomeric modules: Evaluation of mechanical and antibacterial properties. Molecules. 2017; 22:1407-20.

10. Mirhashemi A, Bahador A, Sodagar A, Pourhajibagher M, Amiri A, Gholamrezayi E. Evaluation of antimicrobial properties of nano-silver particles used in orthodontics fixed retainer composites: An experimental in-vitro study. Journal of Dental Research, Dental Clinics, Dental Prospects. 2021; 15:87-93.
11. Ghasemi T, Arash V, Rabiee S, Rajabnia R, Pourzare A, Rakhshan V. Antimicrobial effect, frictional resistance, and surface roughness of stainless steel orthodontic brackets coated with nanofilms of silver and titanium oxide: A preliminary study. Microscience Research Techecnology. 2017; 80:599-607.
12. Espinosa-Cristóbal L, López-Ruiz N, Cabada-Tarín D, Reyes-López S, Zaragoza-Contreras A, Constandse-Cortéz D, et al. Antiadherence and antimicrobial properties of silver nanoparticles against Streptococcus mutans on brackets and wires used for orthodontic treatments. Journal of Nanomaterials. 2018; 2018:1-12.
13. Bindu S, V Kala Vani S, Nirisha G, Madhuri N, Sai Deepa B, Hemadri S. Evaluation of antibacterial effect of silver nanoparticle coated stainless steel band material – an in vitro study. Orthodontic Journal of Nepal. 2019; 9:13-19.
14. Jasso-Ruiz I, Velazquez-Enriquez U, Scougall-Vilchis R, Morales-Luckie R, Sawada T, Yamaguchi R. Silver nanoparticles in orthodontics, a new alternative in bacterial inhibition: In vitro study. Progress of Orthodontic. 2020; 21:24-31.
15. Pokrowiecki R, Wojnarowicz J, Zareba T, Koltsov I, Lojkowski W, Tyski S, et al. Nanoparticles and human saliva: A step towards drug delivery systems for dental and craniofacial biomaterials. International Journal of Nanomedicine. 2019:14:9235- 257.
16. Mohamed A, Wong K, Lee W, Marizan M, Mohd H, Rosli T. In vitro study of white spot lesion: Maxilla and mandibular teeth. The Saudi Dental Journal. 2018; 30:142- 50.
17. O'Reilly M, Featherstone J. Demineralization and remineralization around orthodontic appliances: an in vivo study. American Journal of Orthodontic and Dentofacial Orthopedic. 1987; 92: 33-40.
18. Shaik Z, Rambabu T, Sajjan G, Varma M, Satish K, Raju V, Ganguru S, Ventrapati N. Quantitative Analysis of Remineralization of Artificial Carious Lesions with Commercially Available Newer Remineralizing Agents Using SEM-EDX- In Vitro Study. J Clin Diagn Res. 2017; 11: ZC20–ZC23.
19. NawrockaA , Piwonski I, Sauro S 4, Porcelli A, Hardan L, Lukomska-Szymanska M. Traditional Microscopic Techniques Employed in Dental Adhesion Research-Applications and Protocols of Specimen Preparation. Biosensors. 2021; 11:408-33.
20. Abou Neel E, Aljabo A, Strange A, Ibrahim S, Coathup M, Young A, Bozec L, and Vivek Mudera V. Demineralization–remineralization dynamics in teeth and bone. Int J Nanomedicine. 2016; 11: 4743–763
21. Siddiqi K, Husen A, Rao R. A review on biosynthesis of silver nanoparticles and their biocidal properties. Journal of Nanobiotechnology. 2018; 16:14-42.
22. Wang X, Ji Z, Chang C, Zhang H, Wang M, Liao Y et al. Use of coated silver nanoparticles to understand the relationship of particle dissolution and Bioavailability to Cell and Lung Toxicological Potential. Small. 2013; 10:385-98.
23. Papaioannou W, Gizani S, Nassika M, Kontou E, Nakou M. Adhesion of Streptococcus mutans to different types of brackets. The Angle Orthodontist. 2007; 77:1090-95.
24. Jing D, Hao J, Shen Y, Tang G, Lei L, Zhao Z. Effect of fixed orthodontic treatment on oral microbiota and salivary proteins. Experimental and Therapeutic Medicine. 2019; 17: 4237-243.