Texila International Journal of Public Health
ISSN: 2520-3134
DOI: 10.21522/TIJPH.2013.12.02.Art034

An *In-Vitro* Comparative Evaluation of Microleakage Beneath Metal Orthodontic Brackets when Bonded using Conventional and Titanium Dioxide Nanoparticle Infiltrated Orthodontic Adhesive Resin

Harsha L, Aravind Kumar Subramanian*
Department of Orthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai–600077, India

Abstract

The objective of this present study was to evaluate and compare the microleakage underneath metal orthodontic brackets when bonded with conventional and Titanium dioxide nanoparticle-infiltrated orthodontic adhesive. Ten human caries-free premolars were extracted atraumatically for orthodontics purposes and were randomly allotted into two groups, Group 1: Conventional orthodontic adhesive resin- An acid-etching adhesive system: Enlight composite (ORMCO) and Group 2: An Experimental acid-etching orthodontic adhesive system that was infiltered with Titanium dioxide. An orthodontic adhesive containing 1% nanoparticle was prepared. The metal brackets were bonded to the teeth using the adhesive group to which they belonged. All specimens underwent thermocycling in deionized water for 1000 cycles with a dwell time of 30 seconds and a transfer time of 0 seconds after being stored at 37°C for four weeks in distilled water. The next step involved 24 hours of submersion in a 0.5% basic fuchsin solution. With a low-speed diamond saw, four parallel bucco-lingual longitudinal sections were cut through the occlusal surface. Two calibrated researchers who were blindfolded were examined with a stereomicroscope at a magnification of 16x. Every section's incisal and gingival margins were measured between the bracket-adhesive and adhesive-enamel interfaces. The collected data were tabulated, and the Shapiro-Wilk test for normality was done. At the enamel adhesive interface and the bracket adhesive interface, brackets bonded with the experimental TiO_2 infiltrated orthodontic adhesive resin had higher mean microleakage scores than brackets bonded with conventional composite. However, this difference was only statistically significant at the enamel adhesive interface ($p > 0.05$).

Keywords: *Adhesives, Nanoparticles, Orthodontic Brackets, Titanium Oxide.*

Introduction

Fixed orthodontic treatment involves the provisional attachment of appliances like orthodontic brackets via adhesive resin cement onto the tooth surface to aid in orthodontic movement of the teeth. Though this is common practice, this increases the risk of plaque accumulation and carious lesions on bonded teeth. It was found that enamel decalcification following orthodontic therapy can be seen in 2-96% of the patients. Over time white spot lesions (WSLs) can also be noted due to an increase in the porosity of the surface. Also impedes oral hygiene maintenance by the patient [1, 2].

Most adhesive cement used has a matrix of *bis*-GMA (*bis*-phenol A glycidyl methacrylate). These are often associated with certain water sorption, solubility, lower DC (degree of conversion) and polymerization shrinkage [3, 4]. The precursors for *bis*-GMA are GMA (glycidyl methacrylate) and BPA (*bis*-phenol-A), BPA has been reported as carcinogenic which stimulates certain cytotoxic responses as skin hypersensitivity reactions, hemolytic

Received: 10.06.2024 ***Accepted: 17.06.2024*** ***Published on: 28.06.2024***
Corresponding Author: aravindkumar@saveetha.com

activity, and necrotic cell death [5]. This in recent years has led to the development of *bis-GMA-free* adhesive resins. The advent of low viscose flowable resin cement, manufactured by reducing the filler content by 75-80% compared to conventional resin composite cement, increases flowability but negatively impacts the mechanical properties. This flowability could assist orthodontic bracket adhesion by contributing to the micro-mechanical attachment to the enamel. However, the analysis of scientific data presents a mixed review of its use [6-8].

The literature provides numerous recommendations to overcome WSLs, like periodic fluoride application, strict oral hygiene regime, and incorporation of anti-microbial agents into the resin cement [9-11]. These measures, though effective, are short-term and may further compromise the mechanical properties of the cement.

The most fascinating and promising technology in material science has been Nanotechnology. It opens up possibilities of producing materials with superior mechanical, chemical, biological and optical properties. Nanotechnology and Nanoparticles (NPs) have been explored in dentistry in the quest of the ideal dental material. The NPs' larger surface area and higher charge density promote efficient bacterial cell contact, which improves antibacterial activity. This has led researchers to explore the potential of NPs with anti-cariogenic capacity to combat the issue of WSLs. NPs as silver (Ag), copper (Cu), gold (Au), zinc oxide (ZnO), titanium dioxide (TiO_2), hydroxyapatite, and silicon dioxide (SiO_2), have been known as surfacing coating agents on brackets or incorporated into adhesive cement for this very purpose [12, 13]. Literature suggests that TiO_2 and Ag NPs have been extensively used against enamel demineralization due to their sensitivity against *Streptococcus mutans* (MS). It is also noted that TiO_2 NPs have compatible optical properties with a higher reflective index and colour stability of the resin cement around the bracket [14, 15]. The incorporation of TiO_2 NPs positively influences various mechanical properties such as shear bond strength (SBS), modulus of elasticity, microhardness and flexural strength. That being said microleakage has been a critical factor in deciding the treatment outcome as this dynamic phenomenon allows fluids, ions, and bacterial to reach the tooth cement interface leading to debonding of the brackets, discolouration of restoration margins, secondary caries, hypersensitivity of the tooth and pulpal injury which has not been extensively explored[16,17]. To better understand the microleakage that occurs when metal brackets are bonded with both standard and titanium dioxide nanoparticle-infiltrated orthodontic adhesive resin, the current in-vitro study examined this issue. The hull hypothesis was that the microleakage of conventional and titanium dioxide nanoparticle-infiltrated orthodontic adhesive resins did not differ noticeably from one another.

Material and Method

The Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, scientific research board provided ethical clearance before the current *in-vitro* study was conducted. (SRB/SDC/ORTHO-2004/21/TH-051). The study follows the CRIS standardized reporting requirements created for in-vitro experiments [18].

Sample Size Calculation

Based on research by Felemban et al., sample size estimation was carried out using the G power software (version 3.1) [19].

Preparation of Nano-Infiltrated Adhesive

In the present research orthodontic adhesive containing 1% nanoparticle was prepared by mixing 4 ml of dichloromethane (DCM) and 0.04g of prepared titanium dioxide nanoparticle in a beaker. To ensure no light could pass through the aforementioned beaker was completely covered using aluminium foil. This was then mixed manually while 4g of commercially available orthodontic adhesive resin (Enlight light-cured adhesive, ORMCO) was added. To ensure homogeneous distribution of the NPs throughout the matrix the beaker was rotated 500 rpm (rotations per minute) for 24 hours using an orbital shaker. This material was then retrofilled into a black syringe to prevent light exposure. [20-22]. Figure 1 shows the steps followed in this research to prepare TiO_2 NPs incorporated resin cement.

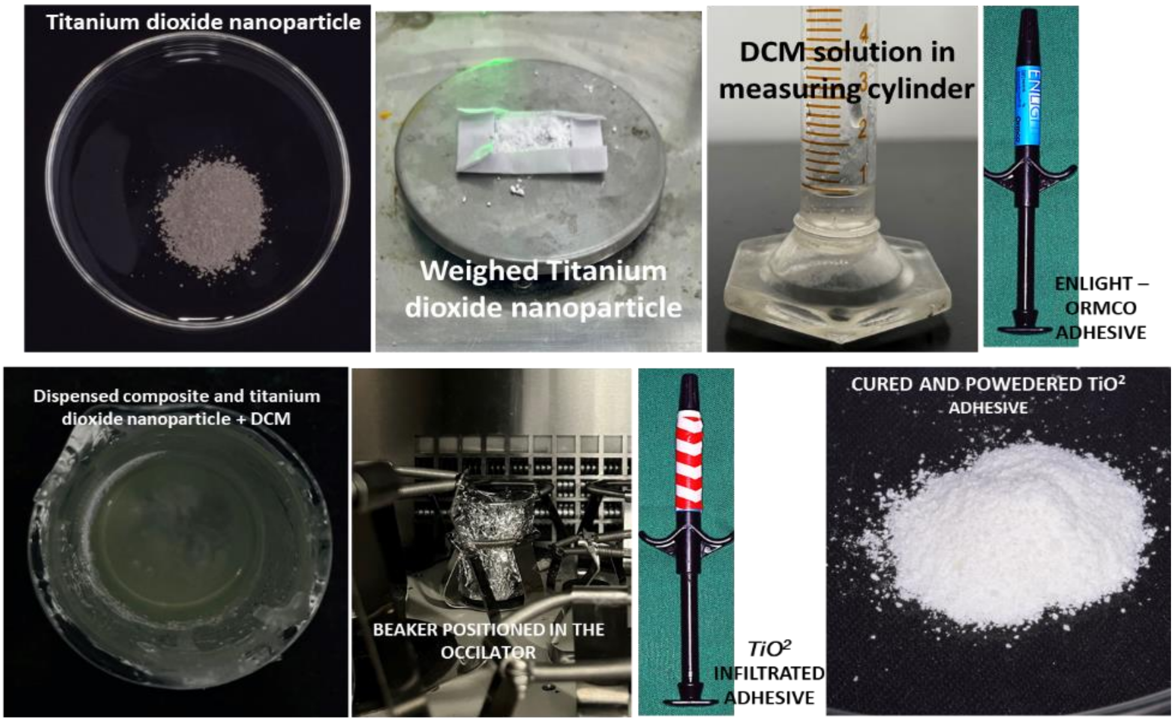

Figure 1. Step-Wise Pictorial Representation of the Prepared Experimental TiO_2 Nanoparticles Infiltrated Orthodontic Adhesive Resin Used in the Research

Sample Preparation, Handling and Allocation

Ten healthy human premolars atraumatically extracted for orthodontic therapy were randomly allotted to two groups of five. The collected premolars were stored in distilled water before they were cleaned, polished for 10 seconds using pumice and a rubber cup and received an adhesive bonding procedure in accordance with the manufacturer. Group 1: Conventional orthodontic adhesive resin- An acid-etching adhesive system: Enlight composite (ORMCO) and Group 2: Experimental acid-etching orthodontic adhesive resin that was infiltered with Titanium dioxide.

All specimens underwent thermal cycling in deionized water for 1000 cycles with a dwell time of 30 seconds and a transfer time of 0 seconds after being stored at 37°C for four weeks in distilled water. Root apices of selected teeth were closed using sticky wax followed by two successive coats of nail paint, 1 mm short of the bracket margins. The next step involved 24 hours of submersion in a 0.5% basic fuchsin solution of all the specimens (Wako Pure Chemical Industry, Osaka, Japan). Before being covered in epoxy resin, specimens were first air-dried and carefully cleaned with distilled water (Struers, Copenhagen, Denmark). With a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL), a total of 4 bucco-lingual longitudinal sections parallel to one another were cut into the occlusal surface. Figure 2 shows the protocol followed in this research for sample preparation and evaluation.

The samples were inspected under a stereomicroscope (Wild Type 308700, Heerbruug, Switzerland) at a magnification of 16 by two calibrated independent blinded researchers. For each of the specimens, scoring was performed on the incisal and gingival bracket borders, between the bracket-adhesive interface and the adhesive-enamel contact. Table 1 lists the evaluation standards that were used; [23].

Table 1. Scoring Criteria for Microleakage [23]

0	There is no dye penetration at either interface.
1	Restricted dye penetration of 1 mm on either interface
2	Penetration of dye up to 2 mm of either interface
3	Penetration of dye up to 3 mm of either interface

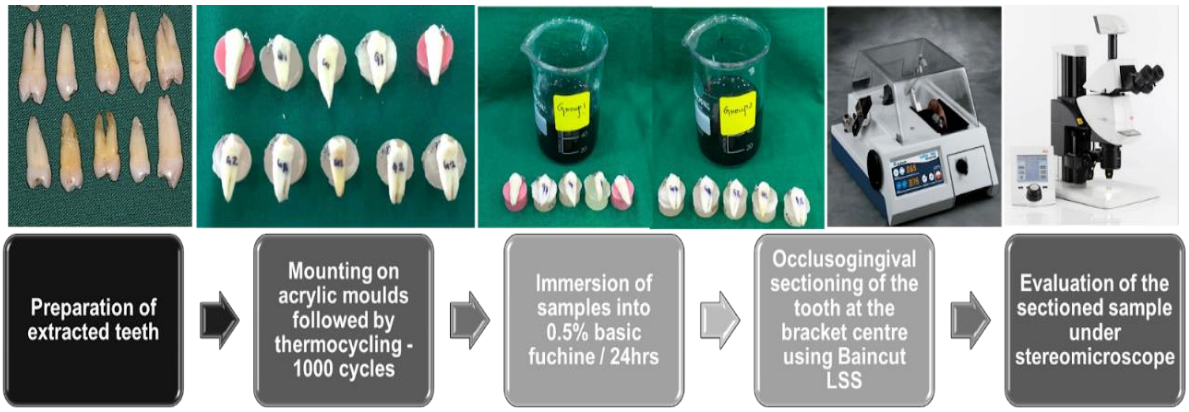

Figure 2. Step-Wise Protocol of Sample Preparation for Evaluation of Microleakage

The mean score of incisal and gingival scores at both the bracket-adhesive and adhesive-tooth interfaces were calculated to obtain the microleakage score for each specimen. The study of microleakage is crucial since it has emerged as one of orthodontics' most difficult subject areas. The involvement of microleakage in bracket debonding and the emergence of white spot lesions is amply supported by the available data. According to literature reviews, the method of curing or the kind of adhesive used does not significantly affect the importance of microleakage.

The information was evenly spread.

Statistical Analysis

SPSS software (Statistical Package for Social Sciences, Version 23.0, Chicago, Illinois, USA) was used to tabulate and analyse the data after it was collected. It was done via descriptive statistics. The Shapiro-Wilk test was used to determine whether the data were normal because the sample size was less than 50 before moving on to the inferential analysis. A normal distribution of the data was observed ($p > 0.05$). To examine intergroup comparisons between groups about mechanical attributes, an independent paired t-test was conducted. statistical significance is indicated when $p > 0.05$.

Results

The mean microleakage for the research groups is shown in Table 2. It should be observed that both groups displayed microleakage at either the adhesive-enamel or the adhesive-bracket interface. While not statistically significant, brackets bonded with TiO_2 infiltrating orthodontic adhesive displayed higher microleakage scores at the bracket adhesive interface than brackets bonded with conventional composite ($p > 0.05$).

When microleakage scores were compared between the adhesive enamel interface, brackets bonded with TiO_2 infiltrating showed a substantial difference in scores. Compared to brackets bonded with standard orthodontic adhesive, orthodontic adhesive displayed higher microleakage (p 0.05). Table 2 displays the statistical data.

Table 2. The Mean Microleakage Scores at Adhesive-Enamel Interface and Bracket-Adhesive Interface From the Incisal-Gingival Side and Overall Comparison Using an Independent T Test between the Two Test Groups

Surface tested	Group	N	Mean ± SD	Sig (2 tailed)
Adhesive enamel interface	G1-Conventional adhesive	5	0.4 ± 0.54	0.004*
	G2-TiO_2 infiltrated adhesive	5	2.2 ± 0.83	
Bracket adhesive interface	G1-Conventional adhesive	5	0.8 ± 1.09	0.108
	G2-TiO_2 infiltrated adhesive	5	2.0 ± 1.0	

*$p < 0.05$- Statistically significant

Discussion

Alkis et al described microleakage "as the passage of fluids and molecules through the tooth-adhesive and adhesive-bracket interfaces, which may cause enamel decalcification, corrosion, discolouration and decreased bond strength". Microleakage is undiscernible clinically till the aforementioned consequences result. Microleakage can significantly diminish the bond strength between the enamel and is stated as the most important reason for bracket debonding[24]. Fixed orthodontic appliance therapy presents challenges in the maintenance of oral hygiene by patients as mechanical plaque removal capability by patients and salivary self-cleaning become less. To prevent enamel decalcification and WSLs topical fluoride application is the first line of treatment.

Topical fluoride may be made available to the patient as gels, pastes, mouth rinses, sealants, varnishes, mouthwashes, and bonding materials for the treatment of WSLs. Most of these formulations are short-term solutions [24-27]. With the dawn of Nanotechnology, the recent decade has seen a significant rise in antimicrobial NPs incorporation in the orthodontics adhesives for prevention of WSLs. Antimicrobials such as NPs exhibit excellent antibacterial properties attributed to the higher surface-to-volume [28].

The incorporation of many NPs has been evaluated for effect on mechanical properties as shear bond strength, optical properties and antimicrobial efficacy. TiO_2 NPs have shown to be potent antimicrobial and significantly reduce the bacterial count against The primary bacteria linked to WSLs, such as early carious lesions, which is thought to be *S.mutans.* Effects on microleakage of TiO_2 incorporation into resin adhesive cement are not well explained in the available literature. The present literature aimed at addressing this lacuna.

The results of earlier studies were carefully considered in the design of the current investigation, and they demonstrated that adding TiO_2 NPs to the orthodontic adhesive resin at a concentration of 1% (w/w) improved the antibacterial effects without deminishing the resin's mechanical qualities [29-31]. The preparation and incorporation of the NPs followed by the established protocol [32] and evaluation also done [23]. They used a stereomicroscope and dye penetration to score the incisal and gingival margins at the bracket-adhesive and adhesive-enamel interfaces. To cut down on mistakes, scoring was done by two impartial, blinded examiners. If the examiners disagreed on the score, they discussed the matter and decided on a final score. The results showed that TiO_2 incorporation at 1% increased microleakage at the adhesive cements enamel interface thereby leading to rejection of the null hypothesis.

Previously *in-vitro* studies were used to evaluate the microleakage of orthodontic adhesive resin [32-35]. Present research as stated above used a dye solution and examination under a stereomicroscope to study the dye penetration [23, 32, 33]. This method is preferred due to its slow cost, non-toxic nature, easy availability of aqueous solutions, quick and direct measurement possibility, the lack of reaction with hard tissues [23, 32, 33, 36].

Table 2 shows that both groups showed microleakage on either of the interfaces evaluated. Higher microleakage score was noted underneath brackets bonded with TiO_2-infiltrated orthodontic adhesive, although statistically not significant, compared to conventional adhesive. When the adhesive enamel interface microleakage scores were examined, a significant difference in scores was found between brackets bonded with TiO_2-infiltrated orthodontic adhesive and brackets bonded with conventional orthodontic adhesive ($p < 0.05$). In contrast to the findings, the gingival margin's microleakage score was higher than the occlusal margin. It was reported that this may be because of the anatomical morphology of premolar teeth, the prominent buccal contour can cause a thicker adhesive layer and higher microleakage score [23]. Additionally, the occlusal site's lower microleakage score was caused by thinner adhesive [37].

According to prior research, the enamel-adhesive interface is more likely to experience microleakage because to its proximity to saliva than bracket-adhesive interactions on both the occlusal and gingival sides. This is contrary to the current studies, and it might be because in-vitro environments use standardised models. Similar findings from in-vitro tests conducted by Yagci et al. and Arhun et al. showed that no statistically significant differences in the microleakage scores of the examined interfaces were found to be [23, 38].

The findings of the current investigation are in agreement with those of the study by Alkis et al, in which it was shown that the enamel-adhesive interface had higher levels of microleakage than the adhesive-bracket interface did [39].

It is vital to note that the significant limitation of the study id its *in- vitro* design which did not entirely reflect the conditions *in-vivo*. The outcomes of the study may be influenced by various factors as oral hygiene practices and dietary intake of the patient, which are crucial in real-life settings. Additionally, the study did not consider saliva, which plays a vital role in oral defence mechanisms. Therefore, future research is needed to evaluate the long-term effects, clinical efficacy and applicability, and mechanical properties of the nanoparticles *in-vivo*. Determining the proper NP particle size, appropriate concentration, and preparation form, such as in addition to primer or adhesive, is highly advised.

One of the drawbacks of this study is the short-term evaluation of the microleakage because brackets typically stay longer intraorally (approximately two years) in clinical settings. For better comprehension, longer evaluation durations with a bigger sample size and assessment of the effect of saliva presence during the bonding on microleakage, we advise further clinical investigations.

Conclusion

Under the limitations of this research, it can be concluded that at the bracket-adhesive interface, no significant difference in the mean microleakage score of conventional and TiO_2 incorporation adhesive resin could be noted. Whereas a significant difference in scores was observed at the Enamel-adhesive interface underneath brackets bonded with TiO_2-infiltrated orthodontic adhesive resin showed more microleakage when compared to brackets bonded with conventional orthodontic adhesive.

Conflict of Interest

None.

Acknowledgement

The authors would like to acknowledge the support received for conducting the study from the Research faculties at Saveetha Dental College and Hospitals, SIMATS, Chennai.

References

[1]. Sruthi, M. A., Gurunathan, D., 2022, An Evidence-Based Classification on the Location of White Spot Lesions in Primary Teeth: A Pilot Study. *World Journal of Dentistry*. Apr 11;13(3):261-5. https://www.wjoud.com/doi/WJOUD/pdf/10.5005/jp-journals-10015-2044

[2]. Verma, P., Jain, R. K., 2022. Visual Assessment of Extent of White Spot Lesions in Subjects Treated with Fixed Orthodontic Appliances: A Retrospective Study. *World Journal of Dentistry.* May;13(3):246. https://www.wjoud.com/doi/WJOUD/pdf/10.5005/jp-journals-10015-2042

[3]. Sunitha, C., Kailasam, V., Padmanabhan, S., Chitharanjan, A. B., 2011, Bisphenol A Release From an Orthodontic Adhesive and Its Correlation with the Degree of Conversion on Varying Light-Curing Tip Distances. *Am J Orthod Dentofacial Orthop,* 140:239-244. https://doi.org/10.1016/j.ajodo.2010.02.037

[4]. Jagdish, N., Padmanabhan, S., Chitharanjan, A. B., Revathi, J., Palani, G., Sambasivam, M., 2009, Cytotoxicity and Degree of Conversion of Orthodontic Adhesives. *Angle Orthod*, 79:1133-1138. https://doi.org/10.2319/080808-418R.1

[5]. Chapin, R. E., Adams, J., Boekelheide, K., Gray L. E., Jr., Hayward S. W., Lees, P. S., 2008, NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Bisphenol A., *Birth Defects Res B Dev Reprod Toxicol,* 83:157-395. https://doi.org/10.2319/080808-418R.1

[6]. Ryou, D-B., Park, H-S., Kim, K-H., Kwon, T-Y., 2008., Use of Flowable Composites for Orthodontic Bracket Bonding. *Angle Orthod,* 78:1105-1109. https://doi.org/10.2319/013008-51.1

[7]. Tecco, S., Traini, T., Caputi, S., Festa, F., de Luca, V., D'Attilio, M., 2005, A New One-Step Dental Flowable Composite for Orthodontic Use: An In Vitro Bond Strength Study, *Angle Orthod*, 75:672-677. https://doi.org/10.1043/0003-3219(2005)75[672:ANODFC]2.0.CO;2

[8]. Uysal, T., Sari, Z., Demir, A., 2004, Are the Flowable Composites Suitable for Orthodontic Bracket Bonding? *Angle Orthod*, 74:697-702. https://doi.org/10.1043/0003-3219(2004)074%3C0697:ATFCSF%3E2.0.CO;2

[9]. Cohen, W. J., Wiltshire, W. A., Dawes, C., Lavelle, C. L., 2003, Long-Term in Vitro Fluoride Release and Rerelease from Orthodontic Bonding Materials Containing Fluoride. *Am. J. Orthod. Dentofac. Orthop*., 124, 571–576. https://doi.org/10.1016/S0889-5406(03)00573-0

[10]. Hussein, F. A.; Hashem, M. I., Chalisserry, E. P., Anil, S., 2014. The Impact of Chlorhexidine Mouth Rinse on the Bond Strength of Polycarbonate Orthodontic Brackets. *J. Contemp. Dent. Pract*., 15, 688–692. https://doi.org/10.5005/jp-journals-10024-1600

[11]. Chung, S. H., Cho, S., Kim, K., Lim, B. S., Ahn, S. J., 2017. Antimicrobial and Physical Characteristics of Orthodontic Primers Containing Antimicrobial Agents. *Angle Orthod*., 87, 307–312. https://doi.org/10.2319/052516-416.1

[12]. Duraisamy R, Ganapathy D, Shanmugam R., 2021. Nanocomposites Used In Prosthodontics and Implantology-A Review. *International Journal of*

Dentistry and Oral Science. Sep 21;8(9):4380-7. https://d1wqtxts1xzle7.cloudfront.net/73188292/IJDOS_2377_8075_08_9043-libre.pdf?1634724245=&response-content-disposition=inline%3B+filename%3DNanocomposites_Used_In_Prosthodontics_An.pdf&Expires=1719480966&Signature=YBSM9FoV3ZA2lQVIwviVMRgzOPTtGQZUfjRI72fstd~ZJdtYIwWD-oJgubgC-pIpUiGTlefKlM4DrsZFWwAfXFBGLmfyBLDDW~3z1brfYfOYpeL7lvuDSVocTb9MT8Y8KgyxzMM5voQIlHeBIKeVKM-EPeQoTGgcJZ-yc9COPV7e3T8NaqJe4GjtMsW-EiLq8uRTzW9QjatXzISq9bHktyMjTYeMvOnRMxghXrbHpivAO4cus7WMJeOD52HZcDCrUz5KzgvIntmrZT4jUi0FUvTb4-maaWxKsa3uI8gJrVUiIQlAYnfNWPs~9F-8T~M6T6oCjDIJK6xgt8NHJex-Ow__&Key-Pair-Id=APKAJLOHF5GGSLRBV4ZA

[13]. Song, W., Ge, S., 2019. Application of Antimicrobial Nanoparticles in Dentistry. *Molecules*, 24, 1033. https://doi.org/10.3390/molecules24061033

[14]. Chokkattu, J. J, Mary, D. J, Shanmugam, R., Neeharika, S., 2022. Embryonic Toxicology Evaluation of Ginger-And Clove-Mediated Titanium Oxide Nanoparticles-Based Dental Varnish With Zebrafish. *The Journal of Contemporary Dental Practice.* Nov 1;23(11):1158. https://thejcdp.com/doi/JCDP/pdf/10.5005/jp-journals-10024-3436

[15]. Govindankutty, D., 2015. Applications of Nanotechnology in Orthodontics and its Future Implications, *Int. J. Appl. Dent. Sci.*, 1, 166–171. http://www.oraljournal.com/

[16]. Varon-Shahar, E., Sharon, E., Zabrovsky, A., Houri-Haddad, Y., Beyth, N., 2019. Antibacterial Orthodontic Cements and Adhesives: A Possible Solution to Streptococcus Mutans Outgrowth Adjacent to Orthodontic Appliances. *Oral Health Prev. Dent.,* 17, 49–56. https://doi.org/10.3290/j.ohpd.a41983

[17]. Akhavan, A., Sodagar, A., Mojtahedzadeh, F., Sodagar, K., 2013. Investigating the Effect of Incorporating Nanosilver/Nanohydroxyapatite Particles on the Shear Bond Strength of Orthodontic Adhesives. *Acta Odontol. Scand.,* 71, 1038–1042. https://doi.org/10.3109/00016357.2012.741699

[18]. Krithikadatta, J., Gopikrishna, V., Datta, M., CRIS Guidelines (Checklist for Reporting In-vitro Studies): A Concept Note on the Need For Standardized Guidelines for Improving Quality and Transparency in Reporting In-Vitro Studies in Experimental Dental Research. *J Conserv Dent*. 2014 Jul;17(4):301-4.

[19]. Felemban, Nayef & Ebrahim, Mohamed., 2017, Effect of Adhesive Layers on Microshear Bond Strength of Nanocomposite Resin to Dentin. *J Clin Exp Dent.*, 9(2):e186-90. https://doi.org/10.4317%2Fjced.53133

[20]. Behnaz, M., Dalaie, K., Mirmohammadsadeghi, H., Salehi, H., Rakhshan, V., Aslani, F., 2018. Shear Bond Strength and Adhesive Remnant Index of Orthodontic Brackets Bonded to Enamel Using Adhesive Systems Mixed with TiO_2 Nanoparticles. *Dent. Press J. Orthod*., 23, 43.e1–43.e7. http://old.scielo.br/scielo.php?script=sci_arttext&pid=S2176-94512018000410001

[21]. Farzanegan, F., Shafaee, H., Darroudi, M., Rangrazi, A., 2021. Effect of the Incorporation of Chitosan and TiO_2 Nanoparticles on the Shear Bond Strength of an Orthodontic Adhesive: An in Vitro Study. *J. Adv. Oral Res.*, 12, 261–266. https://doi.org/10.1177/23202068211015447

[22]. Felemban, N. H., Ebrahim, M. I., 2017. The Influence of Adding Modified Zirconium Oxide Titanium Dioxide Nano-Particles on Mechanical Properties of Orthodontic Adhesive: An in Vitro Study. *BMC Oral Health*, 17, 43. https://doi.org/10.1186/s12903-017-0332-2

[23]. Arhun, N., Arman, A., Cehreli, S B., Arikan, S., Karabulut, E., Gulsahi, K., 2006. Microleakage Beneath Ceramic and Metal Brackets Bonded with a Conventional and An Antibacterial Adhesive System. *Angle Orthod*. November; 76 (6): 1028–34. https://doi.org/10.2319/101805-368

[24]. Cacciafesta, V., Sfondrini, M F., De Angelis, M., Scribante, A., Klersy, C., 2003. Effect of Water and Saliva Contamination on Shear Bond Strength of Brackets Bonded with Conventional, Hydrophilic, and Self-etching Primers. *Am J Orthod*

Dentofacial Orthop. Jun;123(6): 633-40. https://doi.org/10.1016/S0889-5406(03)00198-7
[25]. Asiry, M. A., Alshahrani, I., Alqahtani, N. D., Durgesh, B., 2019. Efficacy of Yttrium (Iii) Fluoride Nanoparticles in Orthodontic Bonding. *J. Nanosci. Nanotechnol.*, 19, 1105–1110. https://doi.org/10.1166/jnn.2019.15894
[26]. Amaechi, B. T., Mcgarrell, B., Luong, M. N., Okoye, L. O., Gakunga, P. T., 2021. Prevention of White Spot Lesions around Orthodontic Brackets using Organoselenium-Containing Antimicrobial Enamel Surface Sealant. *Heliyon,* 7, 3. https://doi.org/10.1016/j.heliyon.2021.e06490
[27]. Hu, H., Feng, C., Jiang, Z., Wang, L., Shrestha, S., Su, X., Shu, Y., Ge, L., Lai, W., Hua, F., 2019. Effectiveness of Remineralising Agents in Prevention and Treatment of Orthodontically Induced White Spot Lesions: A Protocol for a Systematic Review Incorporating Network Meta-Analysis. *Syst. Rev.*, 8, 339. https://doi.org/10.1186/s13643-019-1253-8
[28]. Yun, Z., Qin, D., Wei, F., Xiaobing, L., 2022. Application of Antibacterial Nanoparticles in Orthodontic Materials. *Nanotechnol. Rev.*, 11, 2433–2450. https://doi.org/10.1515/ntrev-2022-0137.
[29]. Assery, M., Ajwa, N., Alshamrani, A., Alanazi, B., Durgesh, B., Matinlinna, J., 2019. Titanium Dioxide Nanoparticles Reinforced Experimental Resin Composite for Orthodontic Bonding. *Mater. Res. Express*, 6, 125098. https://doi.org/10.1088/2053-1591/ab5a93
[30]. Poosti, M., Ramazanzadeh, B., Zebarjad, M., Javadzadeh, P., Naderinasab, M., Shakeri, M.T., 2013. Shear Bond Strength and Antibacterial Effects of Orthodontic Composite Containing TiO_2 Nanoparticles. *Eur. J. Orthod.*, 35, 676–679. https://doi.org/10.1093/ejo/cjs073
[31]. Heravi, F., Ramezani, M., Poosti, M., Hosseini, M., Shajiei, A., Ahrari, F., 2013. In Vitro Cytotoxicity Assessment of an Orthodontic Composite Containing Titanium-Dioxide Nano-Particles. *J. Dent. Res. Dent. Clin. Dent. Prospect.*, 7, 192–198. https://doi.org/10.5681%2Fjoddd.2013.031
[32]. Ulker, M., Uysal, T., Ramoglu, S I., Ertas, H., 2009. Microleakage Under Orthodontic Brackets Using High-Intensity Curing Lights. *Angle Orthod.*, 79:144–9. https://doi.org/10.2319/111607-534.1
[33]. Uysal, T., Ramoglu, S I., Ulker, M., Ertas, H., 2010. Effects of High-Intensity Curing Lights on Microleakage Under Orthodontic Bands. *Am J Orthod Dentofacial Orthop.*, 138:201–7. https://doi.org/10.1016/j.ajodo.2008.09.032
[34]. Abdelnaby, Y L., Al-Wakeel, E E., 2010. Influence of Modifying the Resin Coat Application Protocol on Bond Strength and Microleakage of Metal Orthodontic Brackets. *Angle Orthod.*, 80:378–84. https://doi.org/10.2319/042109-223.1
[35]. Navarro, R., Vicente, A., Ortiz, A. J, Bravo, L . A., 2011, The Effects of Two Soft Drinks on Bond Strength, Bracket Microleakage, and Adhesive Remnant on Intact and Sealed Enamel, *Eur J Orthod,* 33:60–5. https://doi.org/10.1093/ejo/cjq018
[36]. Uysal, T., Ulker, M., Ramoglu, S. I., Ertas, H., 2008. Microleakage Under Metallic and Ceramic Brackets Bonded with Orthodontic Self-Etching Primer Systems. *Angle Orthod.*, 78:1089–94. https://doi.org/10.2319/100507-481.1
[37]. Ramoglu, S. I., Uysal, T., Ulker, M., Ertas, H., 2009. Microleakage Under Ceramic and Metallic Brackets Bonded with Resin-Modified Glass Ionomer. *Angle Orthod.* Jan;79(1):138-43. https://doi.org/10.2319/102607-508.1
[38]. Yagci, A., Uysal, T., Ulker, M., Ramoglu, S I., 2010, Microleakage Under Orthodontic Brackets Bonded with the Custom Base Indirect Bonding Technique, *Eur J Orthod.*, Jun;32 (3):259-63. https://doi.org/10.1093/ejo/cjp090
[39]. Alkis, H., Turkkahraman, H., Adanir, N., 2015. Microleakage Under Orthodontic Brackets Bonded with Different Adhesive Systems, *Eur J Dent.* Jan-Mar;9(1):117-121. https://doi.org/10.4103/1305-7456.149656