University of Mosul
Al-Rafidain Dental Journal
https://rden.uomosul.edu.iq/

Research Article

Effect of Adding Luteolin Nanoparticles on the Antibacterial and Mechanical Properties of an Orthodontic Adhesive

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Article History
Received: 20 July 2023
Revised: 5 September 2023
Accepted: 20 September 2023
Published online 1 September 2025

How to cite: Wahab HY., Saleem NR., Taqa GA., Shammil AA. Effect of Adding Luteolin Nanoparticles on the Antibacterial and Mechanical Properties of an Orthodontic Adhesive. Al-Rafidain Dent J. 2025;25(2):309-322.

10.33899/rdenj.2023.141963.1217

ABSTRACT: The current study aimed to evaluate the effect of adding Luteolin nanoparticles in two different concentrations (1% and 3%) on the mechanical and antibacterial properties of the Transbond XT Unitek Orthodontic Adhesive. **Materials and Methods:** Thirty extracted human upper premolar teeth were used for the shear bond strength test (SBS). The teeth were randomly divided into three groups, ten teeth each: Control group, Luteolin 1% and Luteolin 3% Nanoparticles (NPs) groups. The mesh of the brackets was covered with transbond or modified adhesives, which were then adhered to the teeth. The brackets were debonded using a universal testing machine, and the adhesive remnant index was checked using a stereomicroscope at a 10X magnification. Thirty composite discs were used for the antibacterial test. ten discs were made from transbond adhesive as a control group, and twenty discs (divided equally) were prepared from orthodontic adhesive modified by incorporating Luteolin 1% and Luteolin 3% nanoparticles. The adhesive's antibacterial activity against Streptococcus mutans and Lactobacillus acidophilus was determined by the disk diffusion technique. Statistical analysis was performed at $P > 0.05$. **Results:** The Control group's shear bond strength was statistically higher than that of the other groups (16.1 MPa for the control group and 12.9 MPa, 10.8 MPa for luteolin 1% and Luteolin 3%, respectively). However, the SBS results of this study demonstrated that the mean of all groups was more than that recommended by Reynolds (5.9-7.8 MPa) in 1975. The disc diffusion method showed that the 3% luteolin-modified adhesive was more effective than the 1% luteolin and control groups, with a larger bacterial inhibition zone. **Conclusions:** The incorporation of Luteolin NPs in orthodontic resin produced an antibacterial effect against Streptococcus mutans and Lactobacillus acidophilus without compromising the shear bond strength.

Keywords: Luteolin; Nanoparticles; Shear bond strength; Trans bond orthodontic adhesive.

INTRODUCTION

The primary goals of orthodontic therapy are to enhance the appearance and function of the teeth, improve psychosocial well-being, and prevent long-term problems associated with malocclusion, such as tooth wear, gingival diseases, and pathologies from impacted teeth [1]. However, the appearance of white spot lesions (WSLs) in the enamel may be a side effect of fixed orthodontic therapy and can be accelerated by plaque and oral bacteria accumulation on the brackets [2]. Maintaining good oral hygiene is very difficult in orthodontic patients; therefore, it is important to implement effective antibacterial agents to avoid new WSL formation or cease the progression of any pre-existing demineralization. In recent years, many anti-caries agents have been suggested to prevent enamel surface demineralization post-orthodontic treatments [3,4]. Among the suggested materials, nanoparticles' application has gained the spotlight because of their significant antimicrobial and appropriate physical properties due to their small size and large surface area [5]. This large surface area and high density help them interact more effectively with the cells of bacteria, consequently increasing the antimicrobial efficacy [6]. By the introduction of nanotechnology in modern dentistry. Several efforts have been made to take advantage of orthodontic bonding. Contemporary approaches are mainly the investigation of the effect of antibacterial agents that were incorporated into orthodontic adhesives or cements, or used for coating orthodontic appliances, to decrease bacterial aggregation [6]. Metallic nanoparticles have been suggested as a valuable resource to combat bacterial biofilm development [7]. Organic nanoparticles could be promising antibacterial alternatives because of their natural origin, broad-spectrum antimicrobial effects, low toxicity, and accessibility at an economical cost. By increasing membrane permeability, reducing enzyme synthesis, or stopping biochemical reactions, natural compounds may simultaneously address many bacterial targets [8]. Many recent studies incorporated natural NPs into orthodontic adhesives such as Chitosan NPs, Propolis NPs, Cinnamon NPs, and Curcumin NPs [9,10,11]. Luteolin is a naturally occurring flavonoid that is found in a variety of medicinal plants and vegetables, including thyme and cabbage [12]. Luteolin has been shown to offer pharmacological activities, including antioxidant, anti-allergic, anticancer, anti-inflammatory, and antimicrobial properties [13]. This study aims to determine the effect of adding different concentrations of Luteolin nanoparticles (1% and 3%) on the mechanical and antibacterial properties of orthodontic Adhesive.

MATERIALS AND METHODS

Preparation of Modified Adhesive

Luteolin pure powder was purchased from Yanhuang Industrial Park (Guanxian, Liaocheng, Shandong, China). The modified adhesive with Luteolin was prepared by using an electrical sensitive balance for precise weighing of Luteolin NPs and the adhesive (Figure 1-2). Two different concentrations, which are 1% and 3% were prepared in a weight-to-weight (w/w) ratio. The precise weight of Luteolin NPs was mixed with the corresponding weight of orthodontic adhesive on a sterile glass slab. The modified adhesive material was manually mixed with a metal spatula in a semi-dark room until the nanoparticles were completely wetted within the adhesive and distributed evenly [14]. The Luteolin-modified adhesive was then moved to a sterile disposable syringe and wrapped with dark-colored tape to prevent direct light exposure.

Figure (1): Luteolin nanoparticles

Figure (2): Sensitive electrical balance

Shear bond strength (SBS) and adhesive remnant index (ARI)

All the tooth samples were stored in a sealed container containing distilled water and 0.1% thymol before use. For preparing teeth samples for the SBS test, the teeth were rinsed with tap water and then cleaned with a soft toothbrush to remove any remnant soft tissue. A plastic ring of polyvinyl chloride (PVC) with dimensions of 20 mm on the outside diameter, 18 mm on the inside diameter, and 30 mm in height was used. The rings were then half-filled with dental stone, and after setting, a sticky wax was used to fix the tooth apex on the stone with the long axis of the tooth oriented so that the buccal portion of the tooth sample is parallel to a flat surface that represents the direction of force application during the SBS test [15]. After that, auto-polymerizing cold-cure acrylic resin was added to fill the PVC rings to the level of the cement-enamel junction (CEJ) (Figure 3).

Figure (3): Shear bond strength test samples.

Then the tooth samples were polished for 15 seconds with fluoride-free pumice paste using rubber prophylactic cups. The enamel surface was etched by applying 37% phosphoric acid etch for 15 seconds, rinsing it for 10 seconds, and gently drying for 10 seconds. The etched surface underwent on a chalky appearance [16]. Then the teeth were covered with a thin coat of Transbond primer (3M Unitek). Stainless steel (SS) Standard Edgewise brackets (Dentaurum, Germany) were used in this study. In Group-I 0.022" the bracket was held by a clamping tweezers and the base of the bracket was coated with a thin layer of the conventional Transbond orthodontic adhesive. Modified Transbond 1% Luteolin-NPs and Modified Transbond 3% Luteolin-NPs were used to bond 0.022" SS brackets in Group II and Group III, respectively. A dental explorer was used to evenly distribute the orthodontic or modified adhesives, then the brackets were positioned 4.5 mm from the occlusal surface on the centre of the buccal surface of the crown of the premolar tooth[17].To standardize the pressure for all specimens, a 200-gram weight was secured to a surveyor (Gerdent, China), the surveyor's arm and directed at a right angle to the bracket slot (Figure 4). With the aid of a sharp dental explorer, the excess resin was removed from the bracket's external edges. Then the curing process began utilizing LED light curing equipment (B-Cure, Guilin Woodpecker Medical Instrument Co., Ltd., China) with a (420-480 nm) wavelength. As mentioned by the B-cure manufacturer in the ortho mode that is unique to B-Cure, the light intensity automatically adjusts to 2000mw/cm^2. The curing light was applied for 40 seconds (10 seconds from each mesial, distal, gingival, and occlusal side) [18]. The samples were stored in a sealed container containing distilled water kept at room temperature.

Figure (4): Gerdent Surveyor with a load of 200 gm. to standardize the pressure for all the specimens.

Shear Bond Strength

A universal testing machine (Gester, China) was used for measuring the SBS test of the samples (Figure 5). The shear force was transmitted to the bracket via a shear blade that had the same width as the bracket at a crosshead speed of 1mm/minute. The force needed to shear the bracket and cause bonding failure was measured in Newtons, and the bond strengths were calculated in Mega Pascals (MPa). All samples underwent the test, and the results were recorded in Newtons and then converted into MPa by the following equation:

$$\text{Shear Bond Strength in Megapascals} = \frac{\text{Debonding force in Newton's}}{\text{Bracket base area}}$$

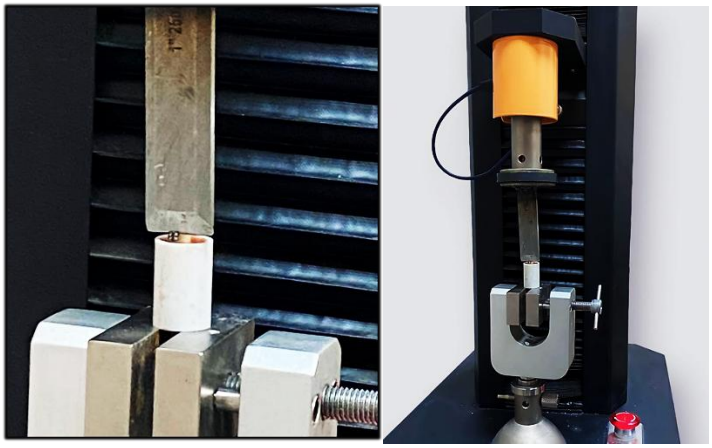

Figure (5): Shear bond strength measurement by the universal testing machine

Adhesive Remnant Index

After debonding of the brackets, all samples for SBS were then tested under the Stereomicroscope (Optika, Italy) at a power of X10 magnification to determine the adhesive remnant index. The following specific scores, as described by Artun and Bergland in 1984, were used to assess the site of bond failure, whether it is cohesive failure, adhesive failure, or mixed cohesive-adhesive failure, and to determine the amount of adhesive material left on the tooth and bracket surfaces:

- Score 0 = No adhesive left on the tooth surface.
- Score 1 = Less than half of the adhesive is left on the tooth surface.
- Score 2 = More than half of the adhesive is left on the tooth surface.
- Score 3 = All of the adhesive left on the tooth, with a distinct impression of the bracket's mesh.

Disc Specimen Preparation

Five strips of Allermine drug were emptied from their tablets and then used as molds for preparing composite discs, molds with a diameter of 7 mm and a thickness of about 2 mm. A total of 30 composite discs were made in these plastic strips; ten discs were prepared using each conventional transbond, modified transbond mixed with 1% luteolin nanoparticle, and modified transbond mixed with 3% luteolin nanoparticles, respectively. The celluloid strips were light-cured for 20 seconds from each side after the molds had been filled with composites (Figure 6). Then, specimens were exposed to UV light (30 min for each side) to make sure there is no contamination [19]. The discs were stored until usage in sealed containers.

Figure (6): Antibacterial test samples

Antibacterial Test

Antibacterial testing was performed against two bacterial strains: *Streptococcus mutans* and *Lactobacillus acidophilus.* Thirty Mueller-Hinton plates were prepared, 10 plates for each group (Control, Luteolin 1% NPs, and Luteolin 3% NPs groups). Five plates of each group were inoculated with 200 µL of bacterial solution for the incubation of the (*Streptococcus mutans*) uniformly by using a sterile swab. The other 5 plates were swabbed with 200 µL solution of the second bacterium (*Lactobacillus acidophilus*) by dipping a sterile swab into the broth and expressing any excess moisture by pressing the swab against the side of the tube. The surface of each agar was completely swabbed and then turned 90 degrees, and the swabbing was repeated. The surface agar was allowed to dry for 5 minutes [20], and then three wells were made in each plate for placing the disc samples by using an empty and sterile insulin syringe, then the discs were gently pressed into the wells using sterile tweezers. After that, the plates were incubated for 48 hours at 37°C. After 48 hours, the zones of inhibition were optically measured with a ruler in millimetres (Figure 7).

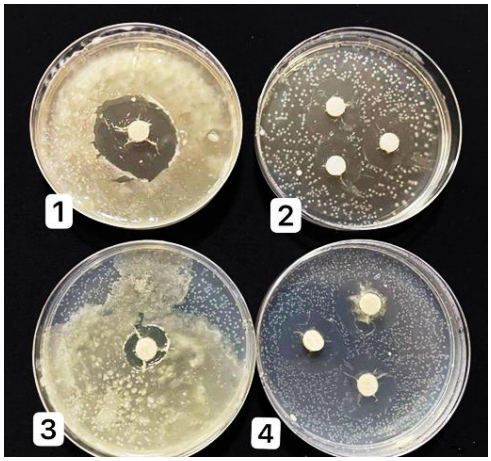

Figure (7): Inhibitory zones in all three groups (1: Luteolin 3%, 2: Luteolin 1%, 3: Control, 4: Luteolin 3%).

Results

All the variables in the present study were checked for their normal distribution by the Shapiro-Wilk test, and it was found that all the groups of SBS and antibacterial sensitivity testing were normally distributed and the parametric tests One way (ANOVA) and Duncan's multiple range test, were used. On the other hand, all the groups of ARI were not normally distributed, and the non-parametric test, Kruskal-Wallis, was used.

The descriptive data of SBS shown in **Table 1** revealed that the control group had the highest mean values of SBS, followed by the luteolin 1% NPs group. While luteolin 3% NPs group showed the lowest mean value.

Table (1): Descriptive statistics for the shear bond strength of the study groups

Groups	N	Mean	Range	Minimum	Maximum	SD
SBS Control	10	16.15980	7.463	12.070	319.53	6.761
SBS Luteolin 1%	10	12.93740	6.490	9.431	15.921	4.142
SBS Luteolin 3%	10	10.88110	6.250	7.990	14.240	3.802

*N: number, SD: Standard deviation

The one-way (ANOVA) statistical test result is illustrated in **Table 2**, demonstrating a significant difference between the mean values of the SBS for the various groups at ($P \leq 0.05$).

Table (2) One-way analysis of variance (ANOVA) for shear bond strength of study groups.

	Sum of Squares	df	Mean Square	F	Sig.
Among Groups	205.896	4	51.474	12.305	.000
Within Groups	188.239	45	4.138		
Total	394.135	49			

df: degree of freedom, **F**: F test, **Sig.**: is significant, Significant level is at (P ≤ 0.05)

Table (3): Multiple Comparisons of the shear bond strength among the study groups using Duncan's Multiple Range Test.

Groups	N	Mean	Ducan Groups
Control	10	16.1598	A
Luteolin 1%	10	12.9374	B, C
Luteolin 3%	10	10.8311	C, D

N: number, * Different litters mean significant difference (P ≤ 0.05).

Adhesive Remnant Index

Kruskal-Wallis Test of ARI

Table (4) displays the results of the Kruskal-Wallis statistical test. This showed a significant difference at (P ≤ 0.05) between the ARI mean scores in this study.

Table (4): Kruskal-Wallis's result of ARI means scores for SBS groups.

Kruskal-Wallis	
Df	**4**
Asymp. Sig.	**0.006**

Df: degree of freedom. **Asymp Sig**: significant level at (P ≤ 0.05).

Table (5): The Adhesive Remnant Index (ARI) scores on enamel tooth surfaces in all three groups.

	ARI Scores			
	0	**1**	**2**	**3**
Control	2	8	0	0
Luteolin 1%	1	4	3	2
Luteolin 3%	1	2	1	6

Descriptive statistics of the antibacterial test

The Descriptive data of the Antibacterial test, as demonstrated in **Table 6,** showed that the Luteolin (3%) group had the highest mean value of the inhibitory zone diameter against the two bacterial strains, followed by Luteolin (1%) and the control group.

Table (6): Descriptive statistics for the inhibition zone of the study groups against bacterial strains.

Groups	N	Mean	Range	Minimum	Maximum	SD
Control	10	18.10000	13.000	10.000	23.000	4.22
Luteolin 1% against *S.mutans*	5	19.00000	9.000	13.000	22.000	3.33
Luteolin 3% against *S.mutans*	5	25.40000	5.000	23.000	28.000	1.95
Luteolin 1% against *L. acidophilus*	5	19.40000	7.000	16.000	23.000	2.67
Luteolin 3% against *L. acidophilus*	5	23.80000	6.000	20.000	26.000	2.14

Analysis of Variance (ANOVA) of Inhibition Zone Diameter

Table (7) illustrates the results of the one-way (ANOVA) statistical test for antibacterial sensitivity, which revealed a significant difference at ($P \leq 0.05$) between the mean values of the inhibition zone diameter for the various groups in the study.

Table (7): One Way (ANOVA) for the mean values of inhibition zone diameters among the study groups

	Sum of Squares	df	Mean Square	F	Sig.
Among Groups	1372.356	8	171.544	24.720	.000
Within Groups	562.100	81	6.940		
Total	1934.456	89			

df: degree of freedom, **F**: F test, **Sig**.: is significant, Significant level is at ($P \leq 0.05$).

DISCUSSION

Orthodontic treatment could cause adverse side effects, including the formation of WSLs in the enamel, which is aided by plaque and oral bacteria accumulation in the bracket [2]. Poor dental hygiene can lead to an increase in the colonization of *Lactobacillus acidophilus* and *Streptococcus mutans*, lowering the pH to a critically low level of 5.5, and promoting the demineralization process, which might lead to the appearance of WSLs. White spot lesions may begin to form one month after the bonding of the brackets in patients with poor oral hygiene, which may compromise their aesthetics [21]. It has been suggested that this problem could be resolved with minimal patient cooperation by adding antibacterial compounds into orthodontic bonding materials [15].

Considering that dental plaque is the primary risk factor for caries and periodontal disease, finding natural products with antibacterial and antiplaque properties could be very beneficial. Many studies suggest that the use of nanotechnology in the management and control of dental plaque biofilms and the remineralization of primary dental caries could result in novel strategies for the prevention and treatment of dental caries [22].

The present study is in agreement with Ahmadi *et al* (2020), who incorporated curcumin (Cur) doped Poly lactic-co-glycolic acid nanoparticles into Transbond orthodontic adhesive and found that adding low concentrations of NPs of Cur-PLGA-NPs did not change SBS significantly in comparison with the Transbond composite (control group) and was within the accepted clinical range [23]. Also, our results are in agreement with Yaseen *et al.* (2020), who modified the orthodontic composite by the incorporation of Nano Cinnamon powder and found that although the shear bond strength of the modified resin was smaller than control, it was higher than the 6 MPa recommended for orthodontic purposes [24].

Regarding ARI, the present study results of control and luteolin 1% groups agree with Poosti *et al* (2013), who reported that following debonding, there was no significant difference between the ARI scores of Transbond alone and Transbond with 1% TiO2[25]. Our results are also in agreement with Farzanegan *et al* (2021), who found that Transbond and Transbond containing 0.5% chitosan NPs + 0.5% TiO2 cause an increase in the failure rate and shift the bond failure towards the composite-enamel interface (ARI score 1) [12].

In regard to the antibacterial test, the result of the present study demonstrated that bacterial biofilm inhibition in modified composites containing Luteolin NPs is significantly higher than in a conventional orthodontic composite. This effect became more obvious as the number of NPs in the composites increased, so that the composite containing 3% NPs considerably reduced *S. mutans* and *L. acidophilus.* The present study results are in agreement with Sodagar *et al* (2016), who incorporated chitosan nanoparticles into a transbond composite and found that the modified composite improved the antibacterial properties without compromising the shear bond strength [15]. This study result is also in agreement with Sodagar *et al* (2019), who added Propolis nanoparticles PrpNPs in different concentrations into transbond XT composite and concluded that nanoadhesives had a significant antimicrobial effect against *S. mutans* and *L.acidophillus* without affecting the shear bond strength [13].

CONCLUSIONS

Within the limitations of the current study, it is possible to conclude that:
The incorporation of Luteolin NPs into orthodontic resin produced an antibacterial effect against Streptococcus *mutans* and *Lacobacillus acidophilus* without compromising the shear bond strength.

Acknowledgment: This study was supported by the College of Dentistry at the University of Mosul / Iraq

Authors' Contribution

Conceptualization: Wahab HY., Saleem NR., Taqa GA., Shammil AA. Data curation: Wahab HY., Saleem NR., Taqa GA., Shammil AA. Formal analysis: Wahab HY., Saleem NR., Taqa GA., Shammil AA. Funding acquisition: Wahab HY. Investigation: Wahab HY. Methodology Wahab HY. Project administration, Resources, Software: Wahab HY., Saleem NR., Taqa GA., Shammil AA. Supervision: Saleem NR., Taqa GA. Visualization: Wahab HY., Saleem NR., Taqa GA., Shammil AA. Writing–original draft: Wahab HY. Writing–review editing: Saleem NR., Taqa GA. All authors have read and approved the final manuscript.

Funding: This study is self-funded

Ethical statement: The protocol of this study was approved by the Research Ethical Committee at the College of Dentistry, University of Mosul.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Availability of data and materials: All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declaration of Generative AI and AI-assisted technologies

No generative AI or AI-assisted technologies were used in the preparation of this work. The authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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تأثير إضـــافة جســـيمات اللوتيولين والأبيجينين النانوية على الخواص المضـــادة للبكتيريا والميكانيكية للاصق لتقويم الأسنان

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4. وزارة الصحة / مديرية صحة كركوك / المعهد الصحي العالي، العراق

الملخص

الأهداف: هدفت الدراسة الحالية إلى تقييم تأثير إضافة جسيمات اللوتيولين النانوية العضوية بتركيزين مختلفين (١٪ و ٣٪) على الخواص الميكانيكية و لتحديد فعالية العامل المضاد للمكروبات للاصق التقويمي.**المواد وطرائق العمل**: في هذه الدراسة ، تم استخدام ثلاثين ضاحكًا علويًا بشريًا مقلوعا لاختبار قوة الشد و تم تقسيم الأسنان عشوائياً إلى ثلاث مجموعات ، لكل منها عشر أسنان : مجموعة القياسية ، اللوتيولين ١٪ و اللوتيولين ٣٪ من الجسيمات النانوية و تم اضافة اللاصق التقويمي القياسي او اللاصق التقويمي المعدل الى شبكة الحواصر المعدنية وتثبيتها على سطح العينة. تم استخدام ماكينة القياس العالمية لقياس قوة القص، بينما بقايا المادة اللاصقة فحصت تحت المجهر باستخدام قوة تكبير (١٠) مرات. وتم استخدام ثلاثين قرصًا لاختبار فعالية العامل المضاد للمكروبات، حضرت عشرة أقراص من مادة اللاصق التقويمي القياسي، وعشرون قرصًا من اللاصق التقويمي المعدل بدمج اللوتيولين ١٪ واللوتيولين ٣٪ من الجسيمات النانوية، فحص نشاط المضاد للبكتريا للمواد اللاصقة ضد بكتريا العقدية الطافرة وبكتريا العصية اللبنية الحمضية بواسطة تقنية اختبار انتشار القرص المزدوج. **النتائج**: كانت قوة رابطة القص للمجموعة القياسية أعلى إحصائياً من المجموعات الأخرى. أظهر اختبار انتشار القرص المزدوج أن المادة اللاصقة المعدلة باضافة جسيمات اللوتيولين ٣٪ النانوية كانت أكثر فعالية من ١٪ والمجموعة القياسية مع منطقة تثبيط بكتيرية أكبر. **الاستنتاجات**: إن اضافة جسيمات اللوتيولين النانوية الى اللاصق التقويمي أنتج تأثيرًا مضادًا للبكتيريا ضد بكتيريا *العقدية الطافرة* و *بكتيريا العصية اللبنية الحمضية* دون التأثير على قوة الشد لمادة اللاصق التقويمي.

الكلمات المفتاحية: إطلاق الفلورايد؛ لاصق تقويم الأسنان؛ قابلية إعادة الشحن؛ قوة ربط القص.