Original Article

Investigation of the Effect of Clove Nanoparticles on Antibacterial Features and Shear Bond Strength of Orthodontic Adhesive Bonded to Human Premolars Teeth

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Abstract

Objectives: The study examined the antibacterial properties of orthodontic composites containing clove as nanoparticles and their mechanical properties after utilizing them to attach brackets to human teeth. **Methodology:** We randomly divided 75 premolars into five groups (n=15). Clove-NPs were prepared and added with percentages of 1%, 2%, 5%, and 10% to the orthodontic composite, the control group without adding. Brackets were bonded to the premolars. The force required for debonding the brackets was measured. After dislodgement, the adhesive remnant index was evaluated. In the microbial test, 135 composite discs were prepared for "the elution component test, disc agar diffusion test, and biofilm inhibition test" to investigate the colonies of bacteria on 3, 10, and 20 days and measure the inhibition growth zone millimeters and efficacy of solutions that may have clove nanoparticles consequently. The studied bacteria included "Streptococcus mutans, Streptococcus sanguinis, and Lactobacillus acidophilus." **Results:** The groups had significant differences between them at the SBS test; the lowest was the 10% CNPs percentage group. According to the DAD test, S. mutans and S. sanguinis had inhibition in growth at all concentrations except 0% and 1%. The development of the Lactobacillus inhibition zone occurred at only 5% and 10% concentrations. Results of eluted samples showed that the lowest numbers of all bacterial colonies were observed on day 20 for the 2%, 5%, and 10% groups; the decrease started on day 10. According to the biofilm test, the bacterial colonies decreased for the 2%, 5%, and 10% concentration groups, and biofilm prevention occurred. **Conclusion:** Clove nanoparticles will make adhesives more bactericidal and stop plaque and bacterial growth. The average shear bond strength of composites with 2% or 5% nanoparticles is still strong enough.

Keywords: Clove Nanoparticles, Microbial Efficacy, Shear Bond Strength (SBS), Orthodontic Composite.

Introduction

In order to maintain proper oral hygiene while using orthodontic appliances, mouthwash, pastes, and irrigators are all necessary. When appliances are worn, plaque accumulation followed by enamel demineralization and gingivitis is a well-known side effect of orthodontic therapy,[1] as is an increase in total microbial residents as well as a changed microflora.[2]

The growths of some microorganisms, such as Streptococcus mutans, Streptococcus sanguinis, and Lactobacillus acidophilus, have been confirmed around brackets and appliances.[3,4] Various studies with clinical notes have examined the impact of braces on oral bacterial colonisation and biofilm formation.[5,6]

These biofilm-associated microbes may have the

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potential to infiltrate into systemic blood circulation and produce a series of problems.[7] In addition, bacterial adhesion that produces biofilms is a well-known step in the pathogenesis of oral infections.[8]

One of the most common microorganisms found in cariogenic plaque on dental hard tissues is Streptococcus mutans. This microbe exhibits anaerobic action, which generates organic acids, at PH levels lower than 5.5. Lactobacillus acidophilus, which assists in the demineralization of dentinal tissues, is a similar bacterium that is frequently found in cariogenic plaque. Streptococcus sanguinis has caries activity with the same PH level.[9] Streptococcus mutans levels were found to be significantly higher during active orthodontic treatment, while they fell after treatment and during the retention period to acceptable levels.[10] These data imply that the oral microflora of individuals may temporarily change when orthodontic devices are present. The oral flora will be restored to pre-treatment levels after the appliances have been taken out of the patients' mouths.[11]

Many studies have been done to prevent any complications that may happen. Researchers found that the involvement of antimicrobials within the appliances is very beneficial to trap bacterial growth.[12] Materials with bactericides included in the braces composite have been proposed for continuous release of the antibacterial component into the mouth cavity.[13] Recently, there has been a lot of discussion about adding nanoparticles to a composite adhesive to enhance its mechanical and antibacterial qualities.

Nanotechnology is a fast-developing field of modern sciences that relates to the size of particles and the application of them with about 1 to 200 nm. Numerous areas of science and technology, including physics, human diseases, dentistry, and many sites, have included nanotechnology.[14] Nanotechnology has contributed to the enhancement of the properties and functions of materials in medicine, as it can produce preferable functionality, particularly due to the nonmetric size included.[15,16] There are lots of studies about the application of nanotechnology in orthodontics, such as nano-coatings in archwires, nanoparticles in orthodontic adhesives, nanoparticle carriage from the elastomeric ligature, and control of oral biofilm during orthodontic treatment, etc.[17,18]

Adding nanomaterials with antimicrobial efficacy to dental composites without decreasing their mechanical properties is considered a great challenge to producing new materials.

These new materials have antimicrobial activity as well as good mechanical properties, like adequate shear bond strength. Many attempts were made, but no one incorporated herbal nanomaterials that have antimicrobial activity into the orthodontic composite.

Clove plants have been employed as a flavouring ingredient in food. The biological activity of clove plants, including antimicrobial, antifungal, insecticidal, and antioxidant activities, has been proven.[19-21] Also, clove oil and extract have properties to prevent the growth of Gram+ and Gram- microbes and yeast.[22]

The activity of clove essential oil with 25 different microbes' species was investigated by Dormans and Deans to see the antimicrobial effect; their results explain that all tested bacteria were susceptible to the essential oils to varying degrees.[23] It is well known that the phenolic chemicals in cloves can break down proteins and interact with phospholipids in cell membranes to alter their permeability, killing a wide range of microbes.[24,25]

The majority of bacterial pathogens developed antibiotic resistance, which made it impossible to treat the bacterial infection with antibiotics and required the use of alternative medical treatments.[26] Also, manufactured antimicrobial material cannot have a long time of action compared with a long time of orthodontic treatment. As a result, the herbal antibacterial is seen as the best option.[27]

The success of orthodontic treatment is dependent on lowering the risk of cavities while maintaining a strong bracket-tooth attachment during the treatment. This study will be conducted to evaluate the impact of adding many percentage concentrations of clove nanoparticles to a composite used in orthodontics. The study will investigate the antimicrobial activity of the composite and measure the bond strength of the braces bonded by the nano-clove composite to human enamel.

The different amounts of nanoparticles added to the composite were used to study how the new resin behaves mechanically at the shear strength of attachment. We would add 0%, 1%, 2%, 5%, and 10% percentages of nano-cloves to the orthodontic composite. The antimicrobial activity of the nano-cloves composite will be measured against the most common bacteria that produce plaque and dental caries, like "*Streptococus mutants, Streptococus sanguinis, and Lactobacillus acidophilus."*

Method and Material

Preparation of cloves as nanoparticles: Clove nanoparticles were prepared by the ionic gelation method. Clove-NPs were created by making a solution of clove as a gel with sodium TPP. When (+) charged clove pieces interact with (-) charged polyanion Sodium Tripolyphosphate TPP, they form an ionotropic gel. Clove solution was immersed in 2% acetic acid aqueous solutions for 20–24 hours at 25 °C with stirring until a clear solution was achieved. The process was continued until the clove solution was frozen and prepared as a powder.[28,29] The morphology of the nanoclove sample was assessed using a Transmission Electron Microscope TEM. The nano-clove particles proved to be of nanoscale size and had a non-aggregated morphology. According to the data shown in Figure 1, particles have an average diameter within the range of 100 to 200 nanometers.

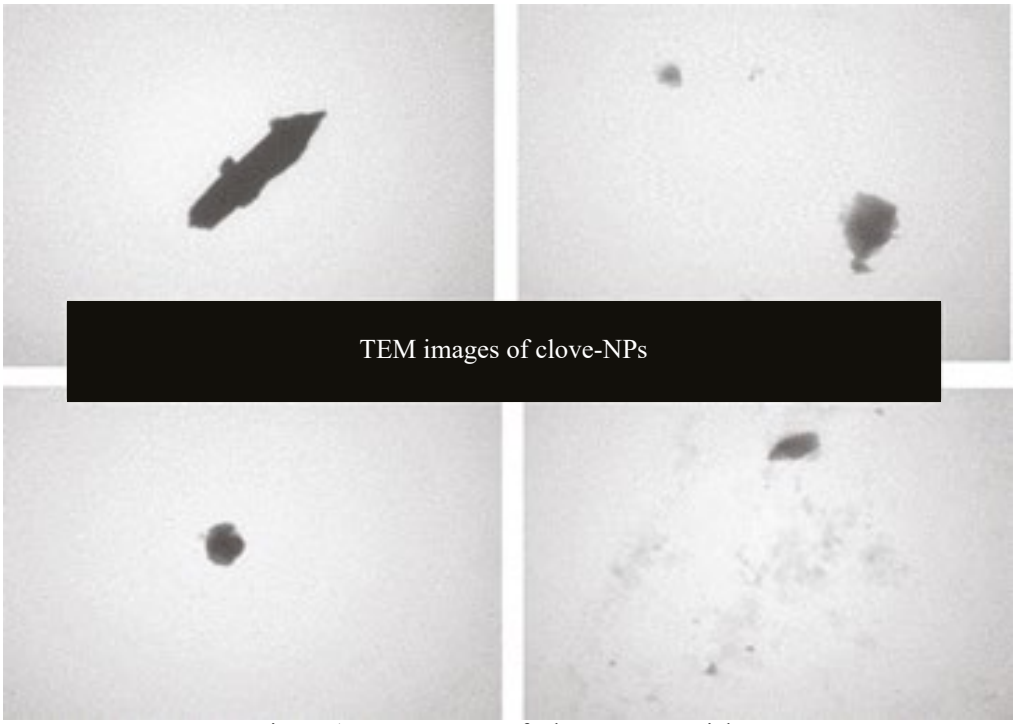

Figure 1: TEM Images of Clove Nanoparticles.

Preparation of composites containing clove nanoparticles: This study has five types of nanocomposites with different concentrations of clove nanoparticles. The first one didn't have any nanomaterials and was used as the control group; the second contained 1% of nanocloves; the third had 2%; the fourth had 5%; and the fifth had 10%. The plain composite that was used for all study groups was the Transbond 3M RESIN composite (3M Unitek, USA). For the production of the nanocomposite with different concentrations of nano-clove, 400mg of clove nanoparticle powder was manually mixed with 3600mg on a dental mixing glass with a dental spoon in a dark area for getting 4000mg of composite ortho with clove nanoparticles at a 10% concentration. To fabricate a material of group 4 with 5% nanocloves, 1200mg of composite with a 10% concentration of nanocloves was mixed with 1200mg of normal Transbond resin. To produce a composite with 2%, nano-clove 80mg of clove nanoparticle was manually mixed powder with 3920 mg of the mentioned plain composite on the slab by a dental spatula. The 1% nanocomposite was fabricated by adding the same amount of 2% nanocomposite to a plain composite and mixing them together to simplify the procedures. The process of mixing proceeded until a uniform colour and texture were achieved. A laboratory scale with an accuracy of 0.0001 gram was used to weigh the plain composite resin and clove nanoparticle powder. The produced nano-composites were stored in a dim environment at 25 °C, re-put in tubes, and labelled with the group name and percentage of nanoparticles before use in the next steps.

Preparation of composite discs: Flat plastic washers were used with equal sizes and parameters for their holes. The washers were put on the surface of the glass of the dental slab. The composite material was flowed inside the rounded holes of the washers, which were 5mm in diameter and about 0.7mm in thickness. The washers were chosen because they are the same size as the orthodontic braces. A smooth, thin glass was put on top of the samples and gently pressed. We ensured that the surfaces of the discs were smooth, without any porosity, and had the same thickness. The light-curing unit was used to cure each sample for 30 seconds. After curing, the composite discs were removed by applying finger pressure to the washers. To eliminate any access, the discs were smoothed and polished with non-fluoride pumas. Afterward, the discs underwent sterilisation through the UV device. By the same procedure, one hundred thirty-five composite discs were prepared for antimicrobial tests. Twenty-seven for each main group of nano concentrations (0%, 1%, 2%, 5%, and 10%) were labelled as mentioned percentages. Each group would be divided into 3 subgroups (n=9) according to the bacteria studied, which were mutans, sanguinis, and acidophilus. Three types of tests were used to check for antibacterial activity: the disc agar (DAD) diffusion test, the eluted component test, and the biofilm inhibition measurement. Three discs were used for each test with different bacteria and different nanoparticle percentages.

Preparation of bacterial suspensions: The three types of bacteria were provided with lyophilized status from the microbial laboratory: "Streptococcus mutans-ATCC25175; Streptococcus sanguinis-ATCC10556; Lactobacillus acidophilus-ATCC4356." These bacteria were incubated in anaerobic broth at 37°C for 48 hours. The concentration of 108 CFU/mL of bacterial suspensions was adjusted

by a spectrophotometer. That means there are about 108 cells per ml.

The "Disc Agar Diffusion (DAD) Test" is a commonly used laboratory method to determine the susceptibility of bacteria to antimicrobial agents, such as nanoparticles. It's a qualitative test that provides information about the effectiveness of different percentages of nanocloves against a specific microorganism. In this test, we evaluated the nanoclove's ability to diffuse within agar around discs, which is known as a microbe's inhibition growth zone. To do this, a medical swapper was used to spread the bacterial suspension, which had 108 CFUs per 1 mL, on the Moller Hilliton agar surface culture medium. Afterward, the nano-composite discs were immersed on the surface of agar at a distance of 2 cm from each other. Then, the culture plate was incubated under growth conditions at 37°C for 72 h. The results were obtained by measuring optically the inhibition of growing zones.

The "Eluted component test" is a laboratory procedure used to assess the effects of nano-clove composite ingredients that are leached or possibly released from discs on microbial growth systems. The discs were immersed in different tubes with 1 ml of sterile normal saline (one tube for each nanoclove concentration). After 72 hours, 50 µL of normal saline was taken from each tube and added to another tube containing 50 µL of microorganism suspension (one tube for each microorganism; the total tubes were 15). The microorganisms were at a concentration of 108 CFU/ml. The tubes were vibrated with a shaking incubator at a speed of 300 rpm for 24 hours at 37 °C. Then the liquid was diluted by using serial-dilution microtiter plates. 10 µL of the diluted liquids were cultured in BHI (Brain Heart Infusion) agar culture mediums and also incubated for one day at 37°. The colonies of the visible microorganisms were estimated using the Miles and Misra method.[30] The same procedure was repeated after 10 days and 20 days by using the same nano-clove discs that were immersed in sterile normal saline in the first step.

The "biofilm inhibition test" is a laboratory experiment used to evaluate a substance's efficacy in preventing the growth or creation of biofilms. Communities of bacteria known as biofilms can be difficult to remove from surfaces and stick to them. We placed the composite discs in a test tube to assess how each microbe used in our study formed biofilms on their disc surfaces. Then, the prepared microbiological suspension was spilled into each tube and incubated for 3 days at 37° to create a biofilm (each microorganism in a separate tube for each clove nanoparticle concentration; total tube n=15). The composite discs were removed after three days using sterile forceps, and any planktonic or loosely attached bacteria were cleaned with a sterilised saline solution. Next, the discs were vortexed in tubes with 1 ml of BHI and sonicated with a sonicator device for one minute. [31] The solution of the tube will be diluted and cultured again in BHI (Brain Heart Infusion) agar culture mediums and also incubated at 37 °C for one day. The formed microorganisms' colonies were counted and calculated using the method of Miles and Misra to get the result of biofilm inhibition of the composite disc. This test was repeated three times with different discs (with the same nanoparticle percentage and bacterial species) to get a more accurate result.

"Evaluation of shear bond strength SBS": For this study, we collected seventy-five premolars that were recently extracted for orthodontics. The teeth were divided into five groups, with fifteen premolars in each group. The selection was done according to inclusion criteria. The tooth's labial faces were etched with 35% phosphoric acid (3M™ ESPE, Scotchbond) for 15 seconds. The teeth were rinsed with flowing water for 20 seconds. The samples were dried using air spray. A tiny layer of primer (3M, Unitek Transpond, USA) was applied and light-cured for 10 seconds. The four types of orthodontic nanocomposite were bonded to the premolar teeth with stainless steel premolar brackets (Advanced, Orthometric, Brazil) based on the group that was related to the sample. For each bracket fixed to the tooth's buccal surface, a force of about 300 g was used to guarantee a homogeneous thickness of the adhesive. A dental probe was used to remove excess resin, and after that, a light-cure device was used to cure the area for 30 seconds. The samples were left in distilled water for one day. The shear bond strength tests had been done on all tooth samples using a universal testing device (Zwick \ Roell Z 050, Germany). The device speed was activated at 1mm per minute perpendicular to the brace's bases. The force necessary for separating the bracket from tooth surfaces was measured in Mega Pascals (MPa) and recorded as the sample SBS shear bond strength value.

"Adhesive remnant index (ARI) evaluation": The premolars and braces were inspected by a stereomicroscope (SMZ 800, Nikon, Japan) at 10x optical resolution to determine how much composite was still present and to determine the scores. The modified ARI scores go from 5 to 1. Score 1: All adhesives and the shape of the brace base were still attached to the teeth. Score 2: More than 75% of the adhesive, but not all of the composite, was still on the tooth. Score 3: The composite is still on the tooth between 25% and 75%. Score 4: The composite on the tooth was less than 25%. Score 5: The tooth surface was free of any composite.

Statistical analysis: The statistical analysis of the data was performed using SAS (Statistical Analysis System, version 9.1). One-way, two-way ANOVA, and least significant differences (LSD) post hoc tests were performed to assess significant differences among means. $P < 0.05$ is considered statistically significant.[32]

Results

Eluted component test: The means and average of bacterial colonies calculated by the eluted component test for all test times are explained in Table 1 and Figure 2.

Table 1: Eluted Component Test Results- the Numbers Multiply by X 10^5.

CFU	Concentration	3 days	10 days	20 days
S. mutans	0%	A96.67±0.88a	B92.33±1.20a	A97.00±1.15a
	1%	A97.00±1.15a	B92.67±1.20a	C81.67±0.88b
	2%	A92.67±1.20b	B82.00±1.52b	C61.67±0.88c
	5%	A81.33±0.88c	B62.67±0.88c	C41.00±0.58d
	10%	A72.00±0.58d	B53.00±1.52d	C23.67±0.88e
LSD		3.86		
S.sanguinis	0%	B82.67±1.45a	B83.33±0.88a	A86.00±1.15a
	1%	A82.00±1.52a	A82.33±1.20a	B71.67±0.67b
	2%	A84.00±0.58a	B72.67±0.88b	C53.00±1.15c
	5%	A71.67±0.88b	B51.67±0.88c	C33.00±0.58d
	10%	A61.67±0.88c	B42.33±1.20d	C11.33±0.88e
LSD		3.47		
L. acidophilus	0%	A94.00±1.15a	A95.67±1.76a	A96.00±1.73a
	1%	A94.67±1.85a	A94.00±0.58a	A94.67±1.73a
	2%	B92.00±1.85a	A96.67±1.45a	B93.33±1.15a
	5%	A93.33±1.15a	B82.33±1.45b	C71.00±0.58b
	10%	A91.33±0.88a	B82.00±0.58b	C42.67±1.20c
LSD		3.31		

Means with a different small letter in the same column are significantly different (P<0.05)
Means with a different capital letter in the same row are significantly different (P<0.05)

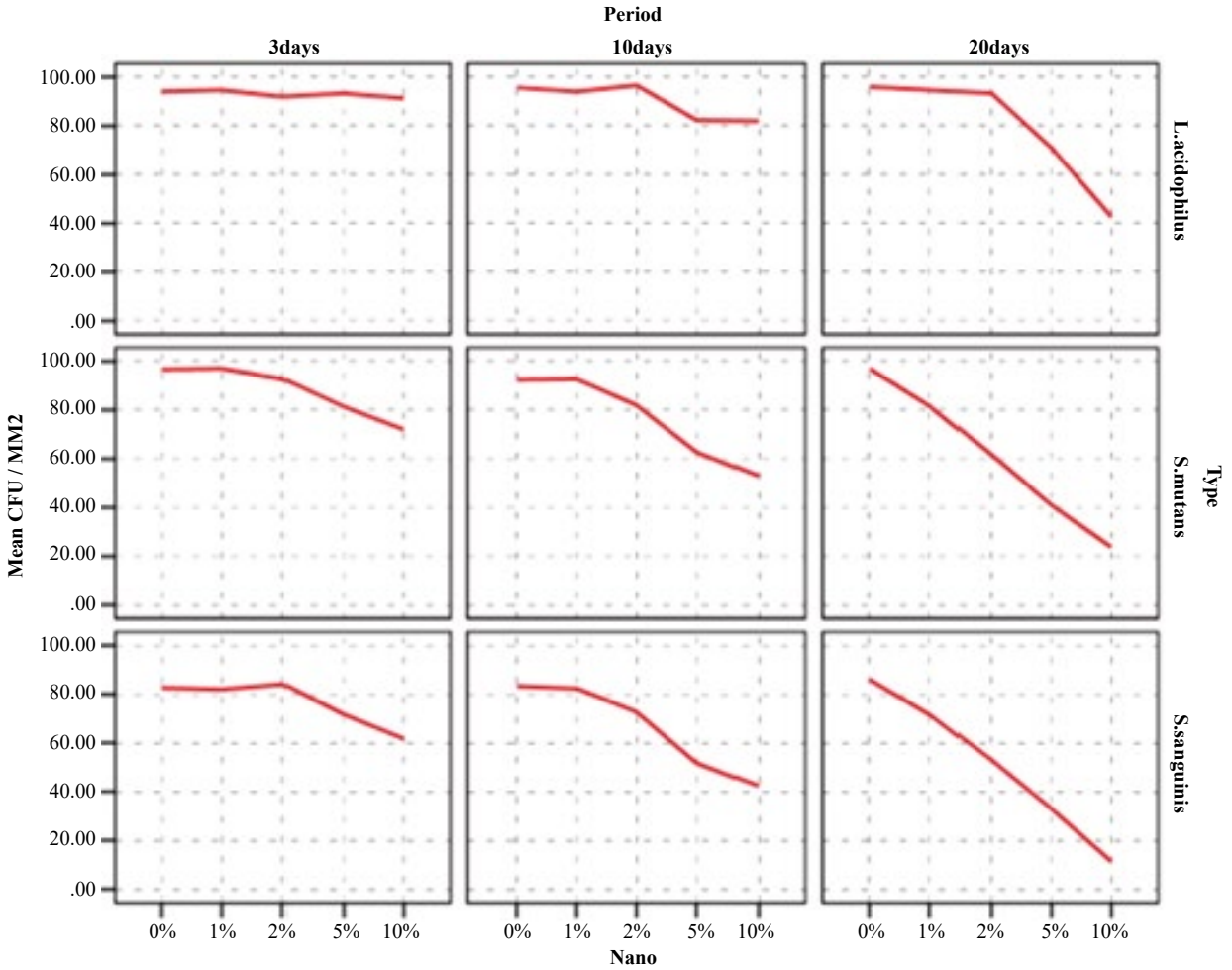

Figure 2: Liner Chart for Eluted Component Test Results.

Disc Agar Diffusion test: The visible inhibition zone diameters were measured in millimeters for the disc agar diffusion test and mentioned in Table 2 and Figure 3.

Table 2: Disc Agar Diffusion DAD Results in Millimeters.

Groups Bacteria	0%	1%	2%	5%	10%
S. mutans	C0.00±0.00a	C0.00±0.00a	B5.67±0.33a	A11.00±0.58a	A11.33±0.33b
S.sanguinis	D0.00±0.00a	D0.67±0.33a	C6.00±0.58a	B11.00±0.58a	A12.67±0.33a
L. acidophilus	C0.00±0.00a	C0.00±0.00a	C0.33±0.33b	B7.67±0.33b	A12.00±0.57ab
LSD	1.05				

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Means with a different capital letter in the same row are significantly different (P<0.05)

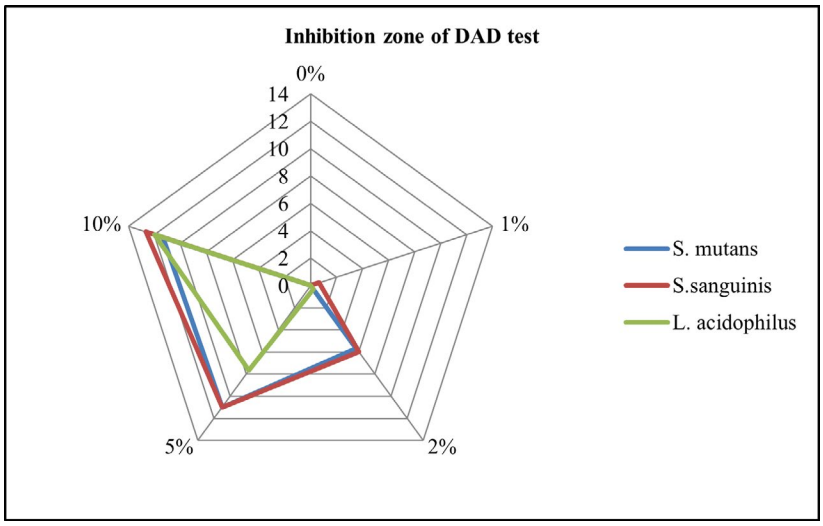

Figure 3: Zone of Inhibition in Diameter. The Number with Percentages Represents the Study Group, and the Number 0 to 15 Represents the Diameters in mm.

Biofilm Inhibition Test: The number of colonies of bacteria that remain after the biofilm inhibition test are mentioned in Table 3 and Figure 4.

Table 3: Biofilm Inhibition Test Result - the Numbers Multiply by X 10^5.

Groups Bacteria	0%	1%	2%	5%	10%
S. mutans	A870.00±66.58a	A873.33±69.36a	B620.00±25.16b	C356.67±17.63b	D133.33±14.52b
S.Sanguinis	A867.00±69.28a	A866.67±52.38a	B656.67±24.03b	C360.00±5.77b	D176.57±18.55b
L.acidophilus	AB833.33±58.11a	AB846.67±31.79a	A896.67±8.81a	B730.00±30.55a	C390.00±11.54a
LSD			116.57		

Means with a different small letter in the same column are significantly different (P<0.05)
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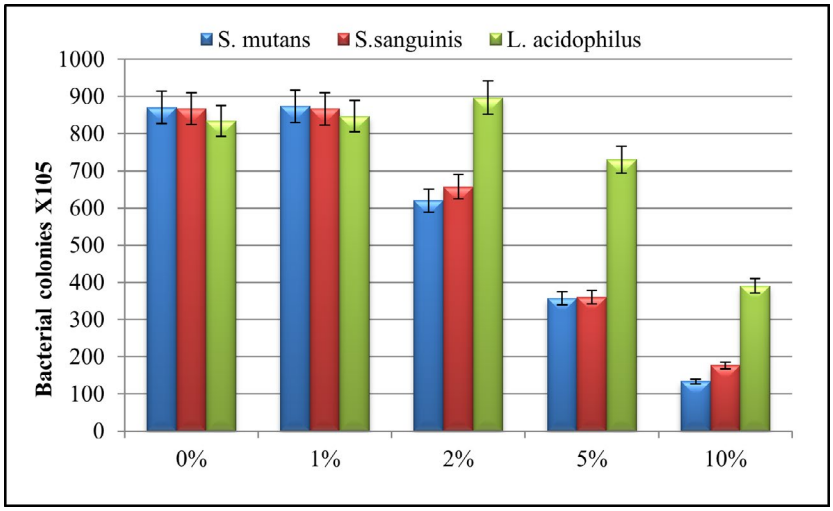

Figure 4: Biofilm Inhibition Test Result.

Shear bond strength test SBS: Results of SBS showed that the differences among all groups were significant (see Table 4 and Figure 5). G represents the study groups. The highest mean was detected for G1(36.20±0.99) followed by G2, G3, G4, G5.

Table 4: SBS Means in MPa Results.

Groups	SBS Mean ± SE
G1	36.20±0.99 a
G2	32.00±1.07 b
G3	27.46±0.89 c
G4	20.93±0.72 d
G5	10.80±0.67 e
LSD	2.49

Means with a different letter are significantly different (P<0.05)

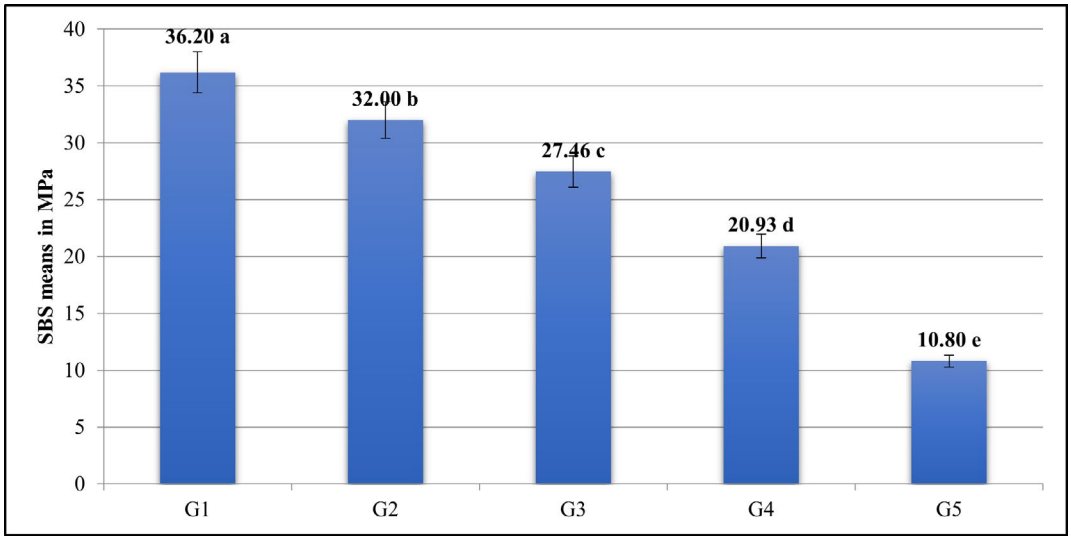

Figure 5: Shear Bond Strength Results.

Adhesive remnant index results: The results of ARI were recorded in Table 5.

Table 5: Modified ARI Results.

Scores Groups	1	2	3	4	5
0%	3	5	3	2	2
1%	4	4	3	3	1
2%	4	3	5	2	1
5%	3	4	5	2	1
10%	5	3	3	2	2

Discussion

For improving the duration of action of the antimicrobial behaviour of dental composite resins, nanotechnology sciences were introduced. Many studies were done with nanoscience to produce composite resins with an antimicrobial effect and acceptable adhesion strength.[33,34] The use of raw materials as nanoparticles will enhance the antimicrobial activity of these materials because the surface area of nanoparticles has intimate contact with composites compared to macro- or micro-particle sizes.[26] Several chemically synthetic antimicrobial materials, such as chlorophenylbiguanid hexane, tetracycline, and many antibiotics, have been made to prevent plaque and treat microbial infections inside the human's mouth.[35] However, these substances have several adverse side effects, including tooth discoloration, oral and intestinal flora disruption, diarrhoea, and many other unfavourable effects.[36]

There is great concern about using herbal materials as antibiotic and antimicrobial agents, which are better than chemical substances. They have minimal side effects on human health and can work for long periods with the original activity. Many medical plants are classified as "GRAS generally recognised as safe," and we can use them for a long time, according to the "FDA/U.S. Food and Drug Administration." One of these medical plants is the clove.[37] Clove oil is frequently employed as a flavouring agent in meals. Scientists are currently studying the potential of using clove as a natural substance to preserve oral hygiene. The focus of the investigation is on its impact on dental plaque, gingivitis, and oral bacteria. In addition, the studies revealed that the mouth rinses mixed with clove exhibited a high reduction of unfavourable bacteria compared to the commonly sold mouth rinses.[38]

Many studies mention the numerous biological effects of clove, which include antibacterial,[20] preventive of yeast growth,[19] and antioxidant[39] behaviors. According to Kanth *et al.*'s[40] research, clove oil was the most efficient product against cariogenic bacteria, with a zone of

inhibition of 30 mm.[40] When Nzeako *et al*.[41] investigated the dental caries-causing microbes using many essential oil extracts, they discovered that clove oil produced the larger inhibition zones.[41]

This study looked at the antimicrobial effect and the bonding strength of Transbond composites mixed with clove NPs. Clove-NPs were added to resins in different amounts (0, 1, 2, 5, and 10%). The main bacteria responsible for dental caries and tooth decay are Streptococcus mutans, Streptococcus sanguinis, and Lactobacillus acidophilus,[42-45] so the study dealt with these bacteria to see the effect of clove on the caries lesion and white spot formation by evaluating the antibacterial effect of clove-NPs. The study's results suggest that the incorporation of nanoparticles (specifically, clove-NPs) into composite resins significantly enhances their ability to inhibit the development of bacterial biofilms as compared to conventional composite resins. The composites with 2% and 5% NPs greatly decreased the growth and adhesions of S. mutans and S. sanguinis, and this impact was stronger as the amount of NPs in the composites rose.

We also found that there was no effect on L. acidophilus proliferation, with discs having a percentage under 5% clove-NPs. Lactobacillus acidophilus helps developing of carious lesions and is also seen in more advanced dental cavitation.[42] Therefore, the biofilm carrying these bacteria was very resistant. When nanomaterials are added at high concentrations, like 5% and 10% groups, we will notice an increase in the antibacterial effect and prevent biofilm formation for all bacteria, including L. acidophilus bacteria. This information is in agreement with Rajini et al. and other previous studies that measured the inhibition of bacterial growth by cloves.[40,41] The results also show the same pattern of incorporating antimicrobial nanoparticles in dental composite resins, like chitosan, ZnO, propolis, Tio2, silver, and Sio2. These studies concluded that the increase in nanoparticle percentage would raise the antimicrobial effect of the composite and increase the inhibition zone area.[46-48]

In the case of alternative nanoparticles, such as Ag, it has been demonstrated that their antibacterial efficacy surpasses that of traditional composites alone when in direct interaction with S. mutans. Nevertheless, there was no significant disparity observed in bacterial proliferation between composites with or without nanoparticles (Ag-NPs) while using Brain Heart Infusion (BHI) medium containing Streptococcus mutans (S. mutans) without direct contact.[49] In contrast, other research has demonstrated the efficacy of composites incorporating nano-silver and nano-silica fillers in preventing enamel demineralization next to orthodontic brackets. The incorporation of silver nanoparticles (NPs) into composites results in a noticeable alteration in colour, specifically a dark grey tint. This phenomenon contradicts the intended aesthetic objectives of the composites.[50-52]

The antibacterial ability of clove nanoparticle ingredients that are released from the orthodontic composite was checked by "the disc agar test (DAD)". The importance of this investigation arises from the observation that demineralization regions commonly develop in close proximity to orthodontic braces. Hence, it is necessary for the orthodontic adhesive to possess antibacterial properties that facilitate its diffusion within the adjacent media. According to the study's conclusions, adding clove nanoparticles to composite discs at concentrations of 2%, 5%, and 10% caused inhibition growth zones for S. mutans and S. sanguinis. The considerable inhibition zones for L. acidophilus were found only in discs above 5% of clove-NPs. This observation suggests that there is a diffusion of clove nanoparticles into the surrounding area, resulting in notable antibacterial activity without direct contact of the composite with bacteria. Our results, like the sensitivity of antibiotics to bacteria in culture media, In "the eluted component test," the antibacterial power of liquids that may contain clove nanoparticles that have been released from composites is measured according to their progress over time. This indicates the continuation of antibacterial activity in the liquid environment. White spot lesions are a persistent issue requiring an antibacterial substance with a prolonged active working period.

The results of this study explained that the lowest bacterial colonies of S. mutan and S. sanguin for the 2%, 5%, and 10% groups were counted on day 20. There was no decrease in colony numbers for the 1% percentage group at that time. The acidophilus colony counts at percentages of 2%, 5%, and 10% NPs decreased significantly at day 20. This measurement referred to a good diffusion of clove-NPs and suitable solubility in the liquid environment. As time passes, the impact of cloves on L. acidophilus will become stronger. These results of the eluted component test are in contrast to the results obtained by adding silver to the composites, which showed a reduction in the colonies of mutan and sanguini only for the 10% nanoparticle. The same results were found in other nanoparticles, such as propolis and TIO2, in comparison to clove nanoparticles.[31,46-48]

Our study determined "the shear bond strength (SBS)" of orthodontic braces under lab-controlled in vitro conditions. Nevertheless, the stresses exerted on the brackets in vivo exhibit some variations. In the oral environment, various forces, including tensile, shear, and rotational forces, will apply to the braces. Furthermore, the mouth cavity involves several variables, including variations in temperature, humidity levels, and the presence of bacterial plaques. These factors all provide challenges in producing laboratory circumstances that closely resemble the complex clinical situations seen in vivo.[53] The recurrent debonding of orthodontic brackets is very undesirable for orthodontic clinics. The debonding and dislodgment of brackets during the treatment period will increase the time of treatment, increase the cost, interrupt the treatment plan, and have a bad impression on the orthodontist.

According to the shear bond strength results, it is clear

that dental composites containing clove nanoparticles show a noticeable decrease as the nanoparticles increase in shear bond strength in comparison to the 0% group. The clove-NPs composite group had the lowest value at 10%, whereas the control group had the maximum SBS range. The obtained shear bond strengths in the 2% and 5% clove nanoparticle groups were still in the range of acceptable clinical values that were concluded by previous studies.[48,54,55] The shear bond strength values for the composite with 2% nanoparticles and 5% groups exhibited minimal variation between them.

Our results of bonding strength are similar to the conclusions of previous studies that mentioned that SBS will significantly decrease when the nanoparticle percentage increases.[48,54] They mentioned the severe reduction of the SBS and debonding threshold of composite resins by adding 10% of other NPs. The adhesive remanent index results showed no significant differences between study groups, which indicated there is no effect regarding the deboned composite from the side of brackets or tooth surfaces.

Conclusion

Clove nanoparticles will make adhesives more antibacterial, which will stop the growth of bacteria and decrease plaque. Composites with 2% or 5% nanoparticles will still have an average shear bond strength that is good for withstanding clinical needs.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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