Original Article

Titanium Dioxide Nanoparticles Enhanced the antibacterial Activity of two Dental Bonding Agents

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Abstract

Objectives: The primary objective of this study is to evaluate the antibacterial efficacy of dental bonding products including titanium oxide nanoparticles. Specifically, we aim to compare the antibacterial activity of these bonding agents with a control group consisting of bonding materials without the incorporation of nanoparticles. The target microorganisms for this investigation are Streptococcus sobrinus and Lactobacillus fermentum. The present study employed a standardised approach to describe the materials and methods used in the research. **Materials and Methods**: Two commercially available universal adhesives, namely Ambar universal (FGM, Brazil) and G-Premio Bond universal (Gc, America), were supplemented with a 4% mass fraction of a nano colloidal dispersion containing titanium dioxide. Initially, sterile discs composed of Whatman no. 1 filter paper were fabricated, with dimensions of 5 mm in diameter and 1 mm in thickness. Subsequently, a total volume of 100 microliters of each bonding agent was employed to fully saturate individual discs, which were subsequently exposed to a 40-second light curing process facilitated by an LED light source. Subsequently, the discs were positioned onto Mueller-Hinton agar. A total of 64 samples were extracted from the adhesives under investigation, and subsequently divided into four groups, each including 16 samples. Two sets of eight samples were collected from each group. Each group was further separated into two subgroups, with each subgroup consisting of eight samples. Subgroup A was designated to assess the antibacterial efficacy specifically targeting Streptococcus sobrinus. Subgroup B is designated for the purpose of evaluating the antibacterial efficacy specifically targeting Lactobacillus fermentum. **Results**: The study findings revealed that the dental bonding agent groups incorporating 4% exhibited considerably greater values for the inhibition zone compared to the control groups. The G-Premio bond universal groups, encompassing both control and integrated groups, exhibited notably larger inhibition zones compared to Ambar universal. **Conclusions**: The addition of titanium dioxide nanoparticles at a concentration of 4% to the universal bonding agents resulted in a notable enhancement of their antibacterial properties when compared to the bonding agents without the incorporation of nanoparticles.

Keywords: Antibacterial Activity, Titanium Dioxide Nanoparticles, *Streptococcus Sobrinus, Lactobacillus Fermentum.*

Introduction

Dental caries is commonly acknowledged as a prevalent chronic ailment that affects persons on a global scale. The facilitation of bonding composite restorations to tooth structure is achieved through the utilisation of adhesive solutions, which commonly do not possess antibacterial qualities. Furthermore, apart from the existence of remaining bacteria within the prepared dental cavity, it is plausible for bacteria to penetrate the tooth via marginal leakage at the adhesive contact, which might potentially result in the formation of secondary caries. In this study, we aim to investigate the effects of social media usage on mental health.

Streptococcus sobrinus and Lactobacilli are gram-positive bacteria that exhibit facultative anaerobic characteristics and are frequently found within the oral cavity of human individuals. Microorganisms exhibit the capacity to metabolise a wide variety of carbohydrates, leading to the generation of a significant amount of lactic acid. The oral hard tissues possess the capacity to stick to surfaces,

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hence creating a notable potential for the creation of biofilms due to their heightened surface energy. The microorganisms examined in this research are widely recognised as being extremely cariogenic and have a substantial impact on the initiation of enamel caries as well as the advancement of secondary caries.[1,2]

The agar diffusion test and the direct contact test are widely utilised procedures for assessing the antibacterial effectiveness of dental materials. The agar diffusion (sensitivity) test is predicated upon evaluating the zones of inhibition that encompass the tested substances. These zones arise due to the diffusion of antibacterial agents released from the bulk of the items under examination. The methodology outlined in this research is commonly utilised as a standard procedure for assessing the antibacterial effectiveness of dental adhesives and restorative materials that have been enhanced with antibacterial agents. The Mitis Salivarius Bacitracin (MSB) agar is frequently utilised for the first isolation and quantification of Streptococcus sobrinus. The composition of this medium consists of mitis salivarius agar that has been added with sucrose, bacitracin, and potassium tellurite. The MRS Agar, alternatively referred to as Lactobacillus selection agar, is generally recognised as a good medium for the isolation and quantification of lactobacilli from several sources, including food, the intestinal system, the vaginal tract, and the oral cavity. The Mueller-Hinton agar is commonly utilised as a medium for assessing the antibacterial effectiveness of adhesives against S. sobrinus and Lactobacilli.

Hence, the objective of this in vitro investigation was to ascertain the potential enhancement of antibacterial efficacy against Streptococcus sobrinus and lactobacillus fermentum by including a colloidal dispersion of titanium dioxide nanoparticles into two universal adhesive systems.

Materials and Methods

Synthesis and Integration of Titanium Dioxide Nanoparticles into Bonding Agents

The present study utilized a patented methodology developed by Cave and Mundell[3], entitled "COATING METAL OXIDE PARTICLES," in order to facilitate the production of a colloidal suspension containing titanium dioxide nanoparticles.

After the colloidal suspension was prepared, 4% of the suspension's mass was added to two universal bonding agents, specifically Ambar universal and G-Premio Bond universal (as shown in Table 1). Each bonding bottle was then subjected to agitation using a mechanical mixing device (Vortex-Mixer; Labnique Inc., USA) for a duration of 2 minutes at a speed of 3000.

Table 1: Chemical composition of the universal bonding agents used in this study

Materials	Composition	pH value
Ambar universal	10-Methacryloyloxydecyl dihydrogen phosphate (10-MDP), bisphenol-A di-glycidyl methacrylate (Bis-GMA) (10–25%), 2-hydroxyethyl methacrylate (HEMA) (10-15%), ethanol (10-25%), water (20%), initiators (1-5%).	3.2
G-Premio Bond universal	Bisacrylamide-1 (25–50%), 10-Methacryloyloxydecyl dihydrogen phosphate (10-MDP) (10–25%), bisacrylamide-2 (2.5–10%), 4-dimethylamino benzonitrile (0.1–1%), Dipenta-erythritol pentacrylate-phosphate (PENTA), Isopropyl alcohol (10–25%), water (20%)	2.5

Preparation of Samples

Initially, sterile filter paper disks (5 mm in diameter and 1 mm in thickness) from Whatman, USA were prepared. Subsequently, a micropipette was employed to apply 100 µL of each adhesive onto individual discs, ensuring their complete saturation. Following the application of light-curing, utilizing a 3M ESPE light source, the discs were promptly positioned onto Mueller-Hinton agar, as described by Amin *et al.*[4], for a duration of 20 seconds.

Distribution of Samples

A total of sixty-four samples were prepared according to the specified procedure from the adhesives under investigation. These samples were then divided into four groups, with each group containing sixteen samples. Subsequently, each group was subsequently divided into two subgroups, each consisting of eight samples, in the following manner.

In this study, a subgroup denoted as Subgroup A was utilized, consisting of a total of eight samples. The primary objective of this subgroup was to assess the antibacterial efficacy specifically against Streptococcus sobrinus.

In Subgroup B, a total of eight samples were employed to assess the antibacterial efficacy against Lactobacillus Fermentum.

Microorganism Isolation

The Preparation of Culture Media with Selective Properties

The Mitis Salivarius Bacitracin Agar (MSB) is a selective medium employed for the cultivation and quantification of Streptococcus sobrinus.

The preparation of the bacitracin stock solution involved the dissolution of 0.364 grammes of bacitracin powder in 100 millilitres of sterile deionized distilled water. To guarantee the complete solubility of the antibiotic, the solution was thoroughly stirred using a magnetic stirrer. The solution underwent sterilisation by means of a Millipore filter featuring a pore size of 0.20 µm, and was subsequently preserved in a refrigerator until its utilisation.[5]

The preparation of the Mitis salivarius medium was obtained by augmenting mitis salivarius agar (MSA) with a 20% (w/v) concentration of sucrose and 200 units/L of bacitracin. Subsequently, the medium is exposed to

autoclaving, a procedure involving the application of a pressure of 15 pounds per square inch at a temperature of 121ºC for a period of 15 minutes. Following then, the medium had a gradual decrease in temperature until it reached an approximate value of 45 ºC. Subsequently, a 1 ml aliquot of bacitracin solution was added to a 1 litre volume of the agar. Subsequently, the resultant mixture was meticulously transferred into Petri dishes possessing a diameter of 90 mm and subsequently allowed to undergo the process of desiccation. According to Golam and Hameed, the Petri dishes were ultimately placed in a refrigerator for storage until they were deemed suitable for use.

The MRS Agar, alternatively referred to as Lactobacillus Selection Agar, is a specialised medium employed for the isolation and quantification of lactobacilli from many sources, including dietary, intestinal, vaginal, and dental flora. The solution is prepared through the dissolution of 65.13 grammes of the substance in 1000 millilitres of distilled water, which is then subjected to boiling until complete dissolution of the medium is attained. A quantity of 1.32 mL of glacial acetic acid was injected into the system and then underwent homogenous mixing. Subsequently, the resultant mixture was put into a culture flask and exposed to a temperature range spanning from 90 to 100 °C for a period lasting between 2 and 3 minutes. The combination is subsequently cooled to a temperature within the range of 45-50 °C. Subsequently, the sample is meticulously moved into sterile Petri dishes, wherein it is allowed to conduct the desiccation process without any disturbance. Subsequently, the desiccated amalgamation is preserved in a light-restricted setting at a temperature ranging from 2 to 8 °C until it becomes suitable for application.

Collection and Culturing of Microorganisms on Selective Media

The microorganisms were cultured directly from swabs obtained from carious lesions of volunteer patients, with an average age range of 12-20 years old. A total of 20 patients were included in the study, with 10 patients selected for the isolation of each microorganism. The samples were collected at the Karbala specialized dental center. According to Gholam and Hameed[6], the swabs were gently rolled across a limited region of the surface adjacent to MSB agar and MRS agar. Subsequently, streaking was performed to isolate microorganisms from the previously inoculated area.

The plates for MSB were subjected to anaerobic incubation using a gas pack for a duration of 48 hours at a temperature of 37 °C. Subsequently, they were incubated aerobically for 24 hours at room temperature. On the other hand, the plates for MRS agar were incubated anaerobically for a period of three days at a temperature of 37 °C.[6] According to Al-Mudallal *et al*.[7], samples were classified as positive if the count of colonies exceeded 250 (104 cells/ml).

Isolation and Identification of Streptococcus Sobrinus Strains

The colonies that were cultivated on MSB-agar medium were subsequently transferred onto newly prepared MSB-agar plates and subjected to anaerobic incubation for a duration of two days. The process of subculturing involved multiple repetitions to achieve the isolation of pure cultures. Al-Jumaily *et al*.[8] conducted an initial identification of S. sobrinus using various methods, including selective agar to observe distinctive colonial morphology, gram staining to determine bacterial cell characteristics, light microscopy to examine cell shape, specific growth characteristics, and sugar fermentation tests for mannitol, sorbitol, sucrose, melibiose, and raffinose.[8]

The microorganism was ultimately identified through the utilization of the BioMérieux VITEK® 2 bacterial identification system, a highly dependable method for discerning bacterial species). A bacterial suspension was prepared in a clear plastic test tube (12x75 mm polystyrene) by inoculating isolated colonies grown on selective media subcultures into 3 mL of sterile saline. The saline used was an aqueous solution containing 0.45% to 0.50% NaCl, with a pH range of 4.5 to 7.0. The turbidity of the prepared suspension was modified to conform to a 0.5 McFarland standard using the VITEK-2 Densi-Check instrument. The duration between the preparation of the inoculum and the filling of the card consistently remained under 30 minutes. The VITEC-2 GP card was employed for the purpose of identifying S. sobrinus.[9]

Identification of Lactobacillus Fermentum

In this section, the focus will be on the identification of Lactobacillus fermentum, a species of bacteria commonly found isolates.

The colonies that were cultivated on a selective medium known as MRS-agar were evenly distributed onto newly prepared MRS-agar plates. These plates were then placed in an anaerobic environment and allowed to incubate for a duration of two days. The process of sub-culturing was iteratively performed to achieve the acquisition of uncontaminated isolates. Subsequently, the cultures were initially classified based on the morphological, cultural, physiological, and biochemical attributes of bacteria. The tests employed in the study included gram reaction, catalase production, cytochrome oxidase/ hydrogen peroxide activity, growth at temperatures of 15 ºC and 45 ºC after one week, acid production from carbohydrates (1% w/v) in MRS broth without glucose, and acid production from beef extract with phenol red as an indicator.[10]

The microorganism was ultimately identified using the BioMérieux VITEK® 2 bacterial identification system, which was employed in a manner consistent with the methodology previously described for the identification of S. sobrinus. The identification of L. fermentum was performed using the VITEK-2 ANC card.2.7. Testing the antimicrobial activity

of the adhesives against the isolated bacteria.

Procedure for Preparing Muller-Hinton agar is as Follows:

The Muller-Hinton agar is a widely utilized medium in internationally recognized protocols for assessing antibiotic susceptibility. The solution was prepared in adherence to the guidelines provided by the manufacturer. Specifically, 38 grams of the substance were dispensed into 1 liter of distilled water. The mixture was subsequently heated to a boiling point to facilitate the dissolution of the powder. To ensure sterility, the solution was then subjected to autoclaving at a temperature of 121 °C for a duration of 15 minutes.

Subsequently, it was securely sealed and stored in a cool, dry location, shielded from intense illumination. The ultimate pH of the medium was determined to be 7.4±0.2. Following the process of medium cooling, it was subsequently transferred into pre-sterilized plastic petri dishes, with a uniform depth of 4 mm. This specific depth aligns with the recommended standard for Muller-Hinton agar when employed in the context of antibiotic susceptibility testing. The bacterial spreading procedures on the Muller-Hinton agar were conducted under sterile conditions using a hood.[6]

Evaluation of the Antimicrobial Properties of the Adhesives

In this study, the antimicrobial efficacy of adhesives containing 4% titanium dioxide nanoparticles was evaluated using the disc method. The adhesives were tested in vitro by direct contact with specific microorganisms, and the results were compared to those of control groups. The disk method is composed of the following steps: Initially, a conventional inoculum of the isolated microorganisms was acquired. The quantification of bacterial populations in a liquid medium can be achieved through a visual assessment of the turbidity of the medium, comparing it to a standardized reference that corresponds to a known concentration of bacteria in a suspension. In this study, the bacterial strains were subjected to standard inoculation procedures. Each bacterial strain was prepared using a 0.5 McFarland Nephelometer Standard, which involves the combination of 0.05 mL of 1.175% barium chloride dihydrate ($BaCl_2 \bullet_2 H_2O$) with 9.95 mL of 1% sulfuric acid (H_2SO_4).[6]

Cultivation

One hundred microliters of each bacterial suspension (Streptococcus sobrinus and Lactobacilli fermentum) were individually extracted using a micropipette and introduced into petri dishes containing Muller-Hinton agar. Subsequently, the bacteria were streaked across the agar surface using a sterile cotton swab, applying gentle pressure in all directions. The adhesive specimens were carefully applied onto the Muller-Hinton agar using sterile tweezers. Each petri dish contained only two adhesive specimens, which adhered effectively to the agar surfaces. Subsequently, the lid of the petri dish was securely sealed, and the dish was placed in an anaerobic environment at a temperature of 37 °C, specifically within an anaerobic jar equipped with gas packs. The study conducted by Kim and Shin[11], employed this technique to evaluate the antimicrobial properties of the adhesive materials when exposed to different bacterial strains.[12]

Quantification of the Zones of Bacterial Inhibition

Following a 24-hour incubation period, the Muller-Hinton agar plates were retrieved from the incubator and subjected to examination in order to determine the presence of an inhibition zone surrounding each disc. The measurement of the inhibition zone was conducted using a ruler positioned around each disc. The measurement of inhibition zones was conducted by two microbiologists in a manner that ensured independence. For each zone, the two microbiologists independently recorded their readings, and subsequently, the mean of their respective readings was calculated. This mean value was then utilized for the purpose of statistical analysis.[12]

Results

Identification Results for the Isolated Bacteria

The laboratory report presents the results of the biochemical test conducted using the VITEK 2 system to identify *Streptococcus sobrinus* bacteria. The test yielded a 94% probability, which the device considers as a highly confident and excellent identification. Similarly, the test also identified *lactobacillus fermentum* with a 91% probability, which is also regarded as a confidently excellent identification by the device.

Inhibition Zones Against S. Sobrinus & L. Fermentum

The inhibition zones for both microorganisms are shown in figures 1 A-B and 2 A-B, respectively, for each of the groups that were tested.

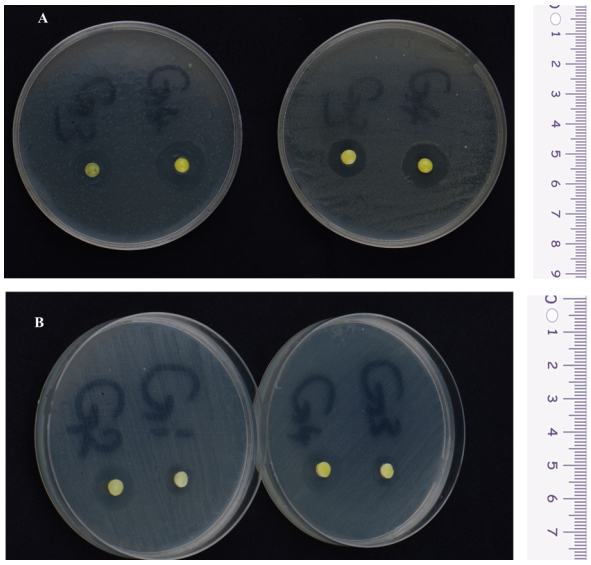

Figure 1: shows some examples of the inhibition zones that were found during the bacteriological testing of adhesives against the bacteria Streptococcus sobrinus and Lactobacillus fermentum.

Descriptive Statistics

Table 2 and Figure 2 (A, B) present the descriptive statistics of inhibition zones for all tested groups against S. sobrinus and L. fermentum, including the minimum, maximum, mean, and standard deviation values. These results can be found in Appendices 11.

Table 2: The present study provides a comprehensive analysis of the descriptive statistical outcomes pertaining to the inhibition zones values observed across all experimental groups in relation to the S. sobrinus and L. fermentum bacterial strains, measured in millimeters

Descriptive statistics		*Streptococcus sobrinus*				*Lactobacillus fermentum*			
Groups	N	Min.	Max.	Mean	Std. Deviation	Min.	Max.	Mean	Std. Deviation
GA	8	5.50	11.50	9.751	0.612	6.50	9.50	8.252	0.454
GB	8	10.60	13.50	11.550	0.425	12.20	13.65	11.544	0.654
GC	8	7.50	9.50	10.535	0.325	8.50	7.50	8.654	0.845
GD	8	11.00	16.00	15.109	0.512	11.60	16.50	13.565	0.785

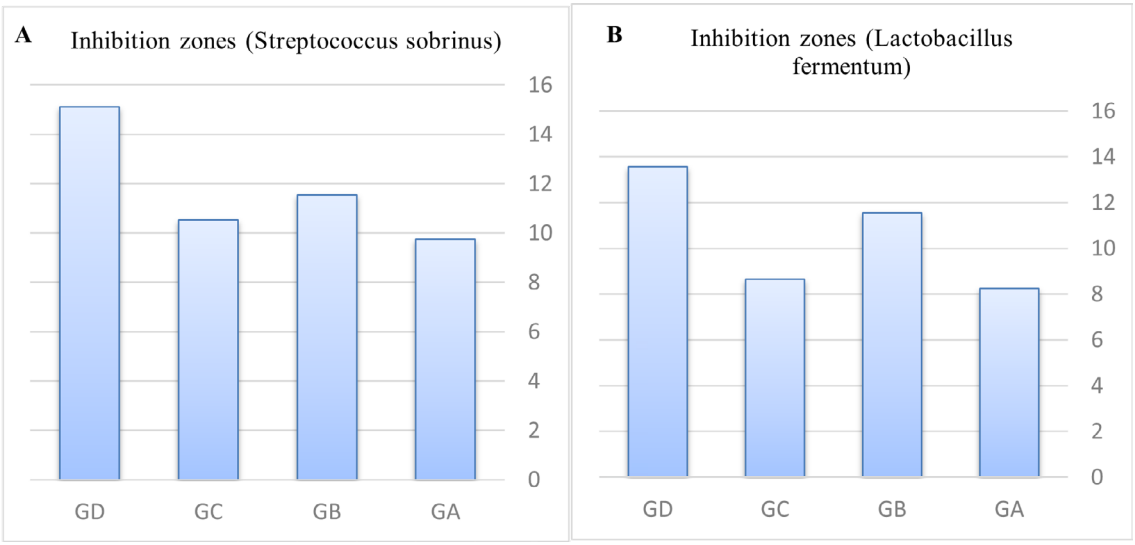

Figure 2: The graph illustrates the average values of inhibition zones for each group in relation to the bacteria S. sobrinus and L. fermentum, measured in millimeters (mm).

Table 2 and figure 2 (A, B) showed that the 4% titanium dioxide nanoparticles incorporated bonding agents (Ambar Universal and G-Premio bond Universal) have greater inhibition zone values against *S. sobrinus* and *L. fermentum* than the control non-incorporated adhesives. The incorporated G-Premio Bond universal have a greater inhibition zone values than Ambar universal adhesives. Ambar Universal adhesive (control) showed the lowest mean values inhibition zones, while the 4% incorporated G-Premio Bond universal showed the highest mean values of inhibition zones. Also, the inhibition zones mean values against the *L. fermentum* are lower than against *S. sobrinus* for all groups.

Inferential Statistics

The findings of the inferential statistics analysis, specifically the independent samples t-test, as presented in Table 3, indicate that the adhesives containing 4% titanium dioxide nanoparticles exhibit significantly greater mean values for inhibition zones compared to the control group. Furthermore, the results of the test indicate that the incorporation of G-Premio Bond universal at a rate of 4% leads to significantly higher values compared to the Amber universal groups.

Table 3: An independent samples t-test was conducted to assess the statistical significance of differences in the mean values of inhibition zones between the adhesives incorporated with 4% titanium dioxide nanoparticles and the control groups, specifically in relation to the bacteria S.sobrinus and L. fermentum

Inferential Statistics t-test	*S. sobrinus*				*L. fermentum*			
Groups	Mean Diff.	Std. Error Diff.	P-value	Sign.	Mean Diff.	Std. Error Diff.	P-value	Sign.
GA vs GB	3.545	0.456	0.000	(HS)	-3.123-	0.454	0.000	(HS)
GC vs GD	3.475	0.215	0.000	(HS)	-3.153-	0.121	0.000	(HS)
GA vs GC	3.785	0.452	0.001	(HS)	-2.454-	0.520	0.000	(HS)
GB vs GD	3.456	0.131	0.002	(HS)	-3.545-	0.451	0.001	(HS)

Discussion

The favourable nature of dental adhesives lies in their capacity to effectively minimise the presence of bacterial biofilm acids and prevent the formation of secondary caries. Furthermore, apart from the existence of bacterial remnants within the prepared dental cavity, it is plausible for bacteria to penetrate through marginal leakage at the limits of the dental restoration, resulting in the formation of secondary caries. Hence, the prospect of dental adhesives exhibiting antibacterial characteristics is attractive in the context of mitigating infections. A multitude of investigations have been conducted to explore the antibacterial characteristics of Titanium dioxide, which have unveiled its capacity to impede the proliferation of diverse microorganisms such as Staphylococcus aureus, Enterococcus faecalis, Streptococcus Sobrinus,[13] Helicobacter pylori, Campylobacter jejuni,[11] Mycobacterium tuberculosis,[14] and certain fungi such as Aspergillus.[13] Furthermore, earlier in vitro studies have shown that Titanium dioxide have the capacity to enhance the inhibitory effectiveness of antibiotics, such as levofloxacin and azithromycin. In a study conducted by Abdulazeem *et al.*[15], an investigation was carried out to assess the antibacterial characteristics of titanium dioxide acid. The study specifically examined the pH-dependent effects of titanium dioxide acid and its targeted actions against different microbes, including Lactobacillus plantarum and E. coli. The study's results indicated that the total suppression of E. coli was observed when a concentration of 60 mM of titanium dioxide was used at a pH of 2.1. The research findings indicate that L. plantarum exhibited total growth suppression, even when subjected to a low quantity of titanium oxide.[15]

Therefore, the objective of this study was to assess the antibacterial effectiveness of adhesives that incorporate 4% titanium dioxide nanoparticles, in contrast to control groups, against the principal etiological agents of dental caries, specifically Streptococcus Sobrinus and Lactobacillus fermentum. The evaluation encompassed the quantification of the areas of inhibition produced by the adhesives in relation to the microorganisms indicated earlier.

The results obtained from this investigation demonstrate that the universal adhesives that were examined displayed diverse levels of microbial inhibitory effects against the microorganisms. The observed results can be attributed to the chemical composition of universal adhesives, particularly the inclusion of HEMA and methacrylated phosphoric acid ester (10-MDP) in Ambar universal, and phosphoric acid modified acrylate resin (10-MDP, PENTA) in G-Premio Bond universal. The veracity of this claim is supported by the results of two distinct research investigations.

In a research conducted by Lukomska-Szymanska *et al.*[16], flow cytometry was utilised to investigate the antibacterial efficacy of adhesives that incorporate phosphoric acid modified acrylate resin (particularly, Gpremio Bond in the Etch&Rinse mode) against E. faecalis. The findings revealed a modest degree of antibacterial efficacy, as 72.04% of the bacterial cells were determined to be nonviable. Lapinska *et al.*[17] conducted an independent study to evaluate the antibacterial effectiveness of different universal adhesives (Adhese Universal, Prime&Bond Universal, and Clearfil Universal) against S. Sobrinus. This evaluation was performed using flow cytometry. The results of the study demonstrated that a substantial number of the adhesives examined had notable antibacterial capabilities. This suggests that these adhesives have the potential to be effective against S. Sobrinus bacteria, which were the subject of the investigation.

However, the findings of this investigation indicate that the use of universal adhesives with a 4% incorporation rate led to a statistically significant increase in the average sizes of bacterial inhibitory zones when tested against Streptococcus Sobrinus and Lactobacillus fermentum, compared to the control groups. The average values of the inhibition zone against Lactobacillus fermentum were observed to be comparatively lower than the average values of the inhibition zone against Streptococcus Sobrinus in all experimental groups. The observed phenomena can be attributed to the elevated acid tolerance displayed by lactobacillus species in comparison to other organisms, leading to their heightened resistance. The increase in the size of inhibitory zones when 4% adhesives containing titanium dioxide are present suggests that the antibacterial characteristics of titanium dioxide are responsible for this phenomenon. In light of the lack of previous scholarly inquiry on the incorporation of titanium dioxide into dental adhesives to assess its antibacterial characteristics, this research aims to indirectly compare its results with previous studies that explored the antibacterial efficacy of titanium dioxide against Streptococcus Sobrinus and other microbial agents. The entire understanding of the antimicrobial and antibiofilm actions of titanium dioxide is still lacking, despite the existence of several postulated ideas. Multiple studies have postulated that the introduction of titanium ions into bacterial cells may result in alterations to their oxido-reduction processes.[13] Consequently, the metabolic activities of bacterial cells can be influenced by the robust antioxidant characteristics exhibited by titanium oxide. An alternative explanation posits that the antibacterial activity could be ascribed to the increased oxidative stressors induced by titanium dioxide. At even minimal quantities, this chemical has the ability to interfere with bacterial biosynthetic pathways that play a vital role in the production and transportation of exopolysaccharides. The process of downregulating and inhibiting biofilm development might result in the increased susceptibility of bacterial cells to external influences, ultimately leading to their mortality.[6,13,15]

The G-Premio Bond universal adhesives demonstrate significantly greater mean values for inhibition zones in comparison to the Ambar universal groups when tested against both types of bacteria. The observed results can be ascribed to discrepancies in the chemical composition and pH levels of the adhesives, with the latter exhibiting a higher acidity.

Conclusion

When compared to their control non-incorporated bonding agents, the antibacterial activity of the two universal bonding agents used in this study was significantly boosted when titanium dioxide nanoparticles were incorporated into the universal bonding agents at a concentration of 4% by mass.

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