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Lactobacillus Reuteri Probiotic Consumption Reduced Various Virulence Gene Expression in Dental Plaque of Fixed Orthodon...

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EDITORIAL

Maria M Marrapodi, Hande Uzunçıbuk, Diana Russo, Silvia Piccolo, Teodoro Solano, Vincenzo Ronsivalle, Gabriele Cervino, Giuseppe Minervini

Technological Advancements in Dentistry: A Step Towards Successful Treatment

[Year:2025] [Month:April] [Volume:26] [Number:4] [Pages:2] [Pages No:331 - 332]

DOI: 10.5005/jp-journals-10024-3869 | Open Access | How to cite |

ORIGINAL RESEARCH

Jasmine Sandhu, Brittany Calderon, Bumsoo Park, Jixa Patel, Franklin Garcia-Godoy, Udochukwu Oyoyo, So Ran Kwon

Zero Waste: Consumers' Perception of the Use of Eco-friendly Toothpaste Tablets—A Quasi-experimental Study

[Year:2025] [Month:April] [Volume:26] [Number:4] [Pages:6] [Pages No:333 - 338]

Keywords: Dentifrice, Environmental sustainability, Patient perception, Toothpaste tablets

DOI: 10.5005/jp-journals-10024-3859 | Open Access | How to cite |

Abstract

Aims: The purpose of the study was to evaluate consumers' perception of the use of eco-friendly toothpaste tablets and evaluate whether there would be a perception difference by consumer demographics: Gender and age. **Materials and methods:** Participants (N = 152) received one packet of toothpaste tablets (Denttabs GmbH, Berlin, Germany). Participants were instructed to brush using toothpaste tablets twice daily. At the end of the 1-week period, participants completed a 10-item questionnaire on their satisfaction of the use of toothpaste tablets. Pearson's Chi-squared test was used to test the distribution of positive and negative responses by gender and by age. Tests of hypotheses were two-sided with α = 0.05. **Results:** The majority responded favorably to cleanliness after usage, flavor, ease of use, importance of eco-friendliness of toothpaste products, intention to switch to tablets, and overall satisfaction with the texture. However, 59.9% of participants disliked the texture of tablets. Based on Pearson's Chi-squared test, there were no statistically significant differences in the distribution of positive and negative responses by gender except for flavor (p = 0.013), where more males responded negatively (27.4 vs 11.5%). Participants were further categorized by age and respective generation (n = 38/age generation type). There were no significant differences in the distribution of positive and negative responses by age generation except for flavor (p = 0.023) and potential switch to toothpaste tablets (p = 0.030). For both items, millennials showed a greater proportion of negative responses than the other generations. **Conclusions:** Toothpaste tablets offer an effective and eco-friendly alternative to traditional toothpaste. While patients generally view them positively, texture remains a major factor influencing acceptance. **Clinical significance:** Consumer responses indicate a positive perception of toothpaste tablets, supporting their potential as a sustainable alternative to conventional dentifrices. Clinicians should highlight both the environmental benefits and the sensory differences when recommending toothpaste tablets to support patient adoption and align with growing sustainability trends.

ORIGINAL RESEARCH

Joko Kusnoto, Siti Sara Safirah, Litayana Ria Anggriani Sitorus, Winnie Valentini, Armelia Sari Widyarman

Lactobacillus Reuteri Probiotic Consumption Reduced Various Virulence Gene Expression in Dental Plaque of Fixed Orthodontic Subjects

[Year:2025] [Month:April] [Volume:26] [Number:4] [Pages:9] [Pages No:339 - 347]

Keywords: Biofilm, Gene expression, Lactobacillus reuteri, Orthodontic, Probiotic

DOI: 10.5005/jp-journals-10024-3857 | Open Access | How to cite |

Abstract

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Aims: The aim of this study was to determine the effect of consuming lozenges containing *Lactobacillus reuteri* probiotic Prodentis lozenges on the expression of *BCR1, ACE2, fadA, aid1, gelE*, and *atlA* genes in dental biofilms of subjects using fixed orthodontic appliances. **Materials and methods:** Plaque samples (n = 20) obtained from a previous study were used in this research. Each subject consumed *L. reuteri* probiotic lozenges (2×10^8 CFU/mL) each day for 2 weeks. RNA was extracted from the samples and synthesized into cDNA. The expression of the gene transcription factors *BCR1*, *ACE2*, *fadA*, *aid1*, *gelE*, and *atlA* genes in biofilms of subjects who used fixed orthodontic appliances was detected using real-time quantitative polymerase chain reaction (RT-qPCR). **Results:** The expression of *BCR1, ACE2*, *fadA*, *aid1*, *gelE*, and *atlA* genes were decreased after consuming the *L. reuteri* probiotic lozenges for 2 weeks ($p < 0.05$). **Conclusion:** Consuming *L. reuteri* probiotic lozenges would affect the expression of *BCR1*, *ACE2*, *fadA*, *aid1*, *gelE*, and *atlA* in plaque from patients using fixed orthodontic appliances. By reducing the expression of the virulence genes, bacterial number would be reduced and biofilm production can also be reduced. **Clinical significance:** Consumption of probiotic lozenges were confirmed to reduce bacterial and fungal biofilm, as proven by the reduction of virulence gene expression. Routine consumption of probiotic lozenges can help reduce potential bacterial infection and increase the oral health of patients using fixed orthodontic appliances.

ORIGINAL RESEARCH

Athanasia E Zarkadi, Despoina Balli, Polyxeni Athanasiadou, Panayioula Lambrou, Aristidis Arhakis, Vasiliki Boka, Konstantinos Arapostathis

Oral Health of Roma Children in Dendropotamos, Greece: A Cross-sectional Study

[Year:2025] [Month:April] [Volume:26] [Number:4] [Pages:8] [Pages No:348 - 355]

Keywords: Cross-sectional, Dental behavior, Dental caries, Oral health, Oral hygiene, Periodontal condition, Roma children

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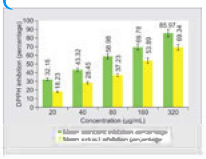
Abstract

Aim: The aim of this study is to document caries status and assess the oral hygiene and periodontal health of Roma children in Thessaloniki-Greece, as well as explore correlations with dental behavior and perceptions. **Materials and methods:** All Roma children attending primary schools in a designated Roma community area of Thessaloniki were examined (*n* = 135). Oral hygiene was evaluated using simplified debris index (DI-S) and simplified calculus index (CI-S), periodontal health was evaluated using the community periodontal index (CPI), and dental caries status was assessed based on ICDAS II criteria. Questionnaires assessed dental behavior and perceptions. **Results:** Approximately 3.7% of individuals were caries-free, whereas 83.0% required restorative treatment. Calculus was found in 54.1% of subjects, and 33% presented with bleeding. A significant 51.9% have never visited a dentist and 40.7% seek dental care only in emergencies. Despite over half (51.1%) expressing dissatisfaction with their dental hygiene, 41.5% reported they rarely brush, 3% used dental floss, and 4.4% used fluoride mouthwash. Furthermore, 10.3% of the subjects reported smoking and 8.9% admitted to alcohol consumption. **Conclusion:** The findings indicate the need to enhance Roma children's oral health awareness and access to dental care. **Clinical significance:** Evaluating the oral health of Roma children identifies unmet needs in a vulnerable group. It enables targeted prevention and treatment strategies. This promotes better overall health and supports equitable healthcare planning and policy development.



ORIGINAL RESEARCH

Priyam Bharathidasan, Priyadharshini Ranganathan, Roohi Singh, Priyanka Barman, Supreet Randhawa, Manvi Chauhan

Phytochemical Screening, Antioxidant, and Anti-inflammatory Activity of Luffa Cylindrica Extract in Oral Carcinoma: An In Vitro and In Silico Analysis

[Year:2025] [Month:April] [Volume:26] [Number:4] [Pages:6] [Pages No:356 - 361]

Keywords: Anti-inflammatory, Antioxidant, DPPH assay, Flavonoids, Luffa cylindrica, Phytochemical, Protein denaturation assay

DOI: 10.5005/jp-journals-10024-3856 | Open Access | How to cite |

Abstract

Aim: This study aims to evaluate the anti-inflammatory and antioxidant effects of *Luffa cylindrica* extract *in vitro* and investigate beta-carotene's molecular interactions with BAX, BCL2, and CDH1 proteins relevant to oral squamous cell carcinoma (OSCC). **Materials and methods:** *Luffa cylindrica* peel was shade-dried, powdered, and extracted with distilled water through heating and filtration. Phytochemical screening was conducted to identify key bioactive compounds. Antioxidant activity was assessed by DPPH scavenging assay at various concentration (20, 40, 80, 160, 320 µg/mL), while anti-inflammatory activity was evaluated via protein denaturation inhibition at various concentration (50, 100, 200, 400, 800 µg/mL). Molecular docking of beta−carotene, a bioactive compound of the extract, with apoptosis-associated proteins BAX, BCL2, and CDH1 was performed using AutoDock 1.5.6, with interaction visualization via BIOVIA Discovery Studio. Statistical analysis was done using one-way ANOVA (SPSS v23), with results expressed as mean ± S.E.M. **Results:** Phytochemical screening revealed the presence of phenols, tannins, terpenoids, and steroids. Antioxidant activity increased dose-dependently, with 69.34% inhibition at 320 µg/mL. Anti-inflammatory analysis showed 85.23% inhibition of protein denaturation at 800 µg/mL. Molecular docking demonstrated strong binding of beta-carotene a bioactive compound of the extract with BAX (−6.8 kcal/mol) and moderate binding with BCL2 (−5.8 kcal/mol), suggesting potential apoptosis-inducing activity. **Conclusion:** *Luffa cylindrica* peel extract exhibits potent antioxidant and anti-inflammatory activities, likely due to its phytochemical profile. The interaction of beta-carotene, a compound from the extract, with apoptotic proteins supports its potential role in anticancer activity. **Clinical significance:** Oral carcinoma requires safer, plant-based therapeutic adjuncts. *Luffa cylindrica* extract showed strong antioxidant and anti-inflammatory effects, while beta-carotene exhibited binding affinity with apoptosis-related proteins. These findings suggest its potential for clinical application in oral carcinoma treatment, warranting further investigation.

ORIGINAL RESEARCH

Mohammad Jalaluddin, Pavithra K Ramanna, Narendra V Penumatsa, Shilpa Mailankote, Deepa Basapur Vijayakumar, Visshishta Jaggannagari

Assessment of the Accuracy and Reliability of the Three Different Devices Measuring Dental Implant Stability: A Comparative Study

[Year:2025] [Month:April] [Volume:26] [Number:4] [Pages:4] [Pages No:362 - 365]

Keywords: Implant stability, Osstell, Osstell Mentor, Periotest, Reliability

DOI: 10.5005/jp-journals-10024-3841 | Open Access | How to cite |

Abstract

Aim: The purpose of this research was to evaluate the accuracy as well as reliability of three distinct devices used to measure dental implant stability. **Materials and methods:** For the current investigation, 45 individuals with one or more missing teeth, aged 18–60 years were enrolled. The implant was inserted into the prepared osteotomy once the osteotomy was completed. Two resonance frequency analysis devices (Osstell, Osstell Mentor) and one damping capacity analysis device (Periotest) were used to measure implant stability. All the participants were evaluated by all the three devices. Readings were obtained at baseline, after 1 month, and after 6 months. Data were recorded and statistically analyzed. **Results:** At baseline, Osstell^{TM} showed a mean stability value of 71.80 ± 4.28, Osstell^{TM} Mentor found a mean stability value of 74.32 ± 3.86 and Periotest was 72.68 ± 2.10. After 1 month, Osstell^{TM} showed a mean stability value of 75.16 ± 2.14, Osstell^{TM} Mentor found a mean stability value of 79.44 ± 2.36 and Periotest was 77.06 ± 1.48. After 6 months, Osstell^{TM} showed a mean stability value of 76.24 ± 1.08, Osstell^{TM} Mentor found a mean stability value of 80.12 ± 1.24 and Periotest was 78.02 ± 1.36. But there was no statistically significant difference obtained between three groups. **Conclusion:** On conclusion, all the three devices used in this study are equally effective. However, when compared to other devices, the Osstell^{TM} mentor is slightly better and more accurate in measuring implant stability. **Clinical significance:** A single indication of implant stability is osseointegration. Assessing implant stability facilitates procedure selection on a patient-by-patient basis, improves case documentation, and aids in decision-making on implant loading. At any point following implant placement, the resonance frequency analysis (RFA) approach offers clinically meaningful data and innovation practices on the condition of the implant–bone interface.

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ORIGINAL RESEARCH

Anas E Alkahlout, Reham I El-Gazzar, Marwa S Shamaa

Effect of Air Abrasion Techniques vs Tungsten Carbide Burs on Enamel Surface after Orthodontic Adhesive Remnant Removal

[Year:2025] [Month:April] [Volume:26] [Number:4] [Pages:7] [Pages No:366 - 372]

Keywords: Adhesive remnant removal, Air abrasion, Aluminum oxide, Enamel surface roughness, Sodium bicarbonate

DOI: 10.5005/jp-journals-10024-3865 | Open Access | How to cite |

Abstract

27

Aim: The study evaluated enamel surface roughness after bracket debonding and adhesive removal using three methods: Tungsten carbide burs (TCB), aluminum oxide air abrasion (AO), and sodium bicarbonate air abrasion (SB). **Materials and methods:** A total of 90 extracted premolars were divided equally into three groups based on adhesive removal methods: TCB, AO, and SB. Procedures were performed under a 4× magnifying loupe. Surface roughness (Ra) was measured using a profilometer and atomic force microscopy (AFM) before bonding (T0). Brackets were bonded, debonded after 24 hours, and the adhesive remnant index (ARI) was assessed. After adhesive removal, Ra was re-evaluated (T1), and removal time was recorded. Data were analyzed using IBM Statistical Package for Social Sciences software. **Results:** The results showed no significant difference in ARI among the three groups. All methods led to a significant increase in Ra after adhesive removal ($p < 0.001$), with the highest Ra in the TCB group, followed by AO, and the lowest in SB. Atomic force microscopy analysis confirmed these findings with surface area roughness (Sa) values. Sodium bicarbonate air abrasion required the shortest adhesive removal time, followed by AO, while TCB took the longest. **Conclusion:** Sodium bicarbonate air abrasion effectively removes adhesive remnants and produces the lowest surface roughness compared with other methods. Air abrasion offers a promising alternative to rotary handpieces, restoring the enamel surface to a nearly original condition and reducing the risk of permanent tooth damage. **Clinical significance:** Sodium bicarbonate air abrasion is a fast, minimally invasive method for adhesive remnant removal. It preserves enamel integrity.

2

ORIGINAL RESEARCH

Harsha Raj BS, Ipsita Jayanti, Asutosh Das, Mayank Trivedi, Reena Chaudhary, Shilpa Duseja

Evaluation of the Effect of Various Surface Conditioning Methods and Attachment of Platelet-rich Fibrin on Dental Implant Surface: An In Vitro Study

[Year:2025] [Month:April] [Volume:26] [Number:4] [Pages:4] [Pages No:373 - 376]

Keywords: Dental implant, Growth factors, Liquid fibrinogen, Osseointegration, Platelet-rich fibrin, Surface conditioning

DOI: 10.5005/jp-journals-10024-3840 | Open Access | How to cite |

Abstract

Aim: The current investigation aimed to assess the impact of three various surface conditioning techniques and platelet-rich fibrin (PRF) attachment on the surface of dental implants. **Materials and methods:** The study employed overall 60 implants, each measuring 13 mm length and 3.75 mm diameter which were divided into three groups (n = 20). Group I: Surface modification using sandblasting and acid etching method, group II: Surface modification using plasma spraying method, and group III: Surface modification using UV light method. To acquire the PRF clots, 9 cc of blood samples were taken to prepare PRF. The samples from each group (20 samples per group in 3 separate containers) were submerged for 60 min at room temperature. A scanning electron microscope with 5000× magnification was employed to evaluate the baseline surface roughness area and PRF attachment on the surface modified implants. All data were gathered and analyzed statistically. **Results:** At baseline, sandblasting and acid etching method mean surface roughness was 0.86 ± 0.02. In plasma spraying group, 0.90 ± 0.10 and in UV light method, the mean surface roughness was 0.84 ± 0.06. No significant difference was obtained. After intervention, the maximum PRF attachment on implant surface was found in sandblasting and acid etching group i.e., 3.02 ± 1.04 followed by UV light group (2.88 ± 0.76) and plasma spraying group (2.20 ± 1.28). A significant difference was obtained between the different methods. **Conclusion:** The present study concluded that, according to the current investigation, all surface changes demonstrate adhesion between the PRF and the implant surface. But the sandblasting and acid etching group exhibited the highest PRF adhesion compared to UV light and plasma spraying groups. **Clinical significance:** The total amount of time between implant insertion and prosthesis delivery is influenced by osseointegration time. Numerous growth factors and inflammatory mediators regulate the complex procedure. In addition to releasing vital growth factors like PDGF and TGF, which are in charge of bone remodeling, PRF can also provide a fibrin scaffold. Therefore, PRF can help promote quicker osseointegration when applied as a surface coating to the implant shortly before insertion.

ORIGINAL RESEARCH

Varalakshmi K Raja, Prema Anbarasu, SP Saravana Dinesh, Saravana Kumar Subramanian, Gabriel Eisenhuth, Sebastian Eisenhuth, Claudia Eisenhuth, Shilpa Bhandi

An Evaluation of the Relationship between Condylar Guidance, Occlusal Plane Orientation, Cuspal Inclination, and Compensatory Curves in Permanent Dentition

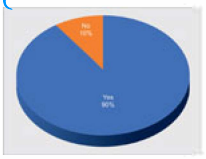
[Year:2025] [Month:April] [Volume:26] [Number:4] [Pages:6] [Pages No:377 - 382]

Keywords: Compensatory curve, Condylar guidance, Cuspal inclination, Occlusal plane, Orthodontics, Temporomandibular joint dysfunction

DOI: 10.5005/jp-journals-10024-3851 | Open Access | How to cite |

Abstract

Background: Understanding the relationship between condylar guidance (CG), occlusal plane orientation, cuspal inclination, and compensatory curves in natural dentition is important in orthodontics for ensuring post-treatment stability and managing temporomandibular joint disorders. Therefore, the study's objective is to assess the relationship between the compensatory curve in natural dentition, the inclination of functional cusps and occlusal plane, and CG. **Materials and methods:** Condylar guidance, occlusal plane and cuspal inclination, and the compensating curve were measured in 56 pretreatment samples (lateral cephalograms and study models). The study models' STL images, digital cephalometric tracing with FACAD software, and 3D scanning technology (Shining 3D Auto Scanner) were used to evaluate the parameters. The associations between cuspal inclination, occlusal plane orientation, CG, and the compensating curve were evaluated using multivariate correlation analysis. **Results:** The analysis revealed an insignificant correlation between CG, occlusal plane and cuspal inclination, and the curve of spee. Furthermore, it shows statistically insignificant relationship, indicating that variations in these factors do not significantly influence each other. **Conclusion:** There is no substantial evidence to suggest a direct correlation between the factors of CG, occlusal plane and cuspal inclination, and the compensatory curve in natural dentition. These findings imply that changes in occlusal morphology and plane inclination during orthodontic treatment do not have a significant impact on CG or post-treatment stability.



2

ORIGINAL RESEARCH

Mohammed Mohsen Al Jearah

Knowledge, Perception and Awareness of Clear Aligner Treatment among General Dentists of Saudi Arabia: A Cross-sectional Study

[Year:2025] [Month:April] [Volume:26] [Number:4] [Pages:5] [Pages No:383 - 387]

Keywords: Awareness, Clear aligners, Knowledge, Questionnaire, Saudi Arabia

DOI: 10.5005/jp-journals-10024-3850 | Open Access | How to cite |

Abstract

Aim and background: The aim of this study was to evaluate the knowledge, perception, and awareness of general dentist of Saudi Arabia in respect to clear aligner treatment (CAT). **Methods:** A cross-sectional study in which a 15-question-based questionnaire was sent to 428 general dental practitioners (GDP) across the region of Saudi Arabia via Google Forms. Five questions each were comprehensively designed to evaluate the knowledge, perception, and awareness of the participants. They were asked to fill the questionnaire and send back the form within 30 days. The data were recorded and statistically analyzed. **Results:** A total of 402 responses were received back with a response rate of 94%. Approximately 91.5% GDP were aware about CAT and 94.7% preferred an orthodontist's opinion before starting case of CAT. Approximately 93.5, 94, and 95% GDP agreed that CAT is more comfortable, less painful and easy to maintain oral hygiene as compared to braces and wires, respectively. Approximately 90 and 45.3% GDP believed that CAT can be used to treat simple and complex cases of malocclusion, respectively. Approximately 71% GDP consider that aligners are less technique sensitive than fixed orthodontics. Approximately 48% GDP believed that aligners must be changed after 2–3 weeks. Approximately 47.5% believed that attachment required on all teeth. Approximately 39% consider aligners as medical waste. **Conclusion:** General dental practitioners are aware about the concept of aligners, its advantages over braces and patient preferences. However, there is scope of further learning and improvement regarding indication, effectiveness, recommendations, and other technicalities of CAT. **Clinical significance:** Clear aligner treatment is gaining rapid recognition owing to its esthetic advantage, digital workflow and comfortability. This study highlights the awareness of dentists about the general understanding of CAT but also throws light on their limited knowledge about the technical aspects of the treatment.

ORIGINAL RESEARCH

Reem M Al Shaibah, Reham I El-Gazzar, Ahmed M Hafez

Comparative Evaluation of Enamel Microfracture and Adhesive Remnant Index of Adhesive Precoated Flash-free System vs Conventional Bonding Using Different Debonding Techniques: An In Vitro Study

[Year:2025] [Month:April] [Volume:26] [Number:4] [Pages:9] [Pages No:388 - 396]

Keywords: Adhesive precoated flash-free adhesive, Adhesive remnant index, Conventional brackets, Debonding instruments, Enamel microcracks

DOI: 10.5005/jp-journals-10024-3858 | Open Access | How to cite |

Abstract

Aims: To evaluate and compare adhesive remnant index (ARI) between adhesive precoated (APC) flash-free (FF) appliance system and conventional brackets using four different debonding techniques, and to assess the relationship between debonding methods and enamel crack formation. **Materials and methods:** A total of 80 sound human premolars were randomly allocated into two main groups (n = 40 each): APC-FF and conventional brackets. Each group was further subdivided into four subgroups (n = 10) based on debonding methods. Weingart plier, Howe plier (HP), straight cutter (SC), and bracket removing plier (BRP). Following standardized bonding protocols, brackets were debonded and evaluated for ARI scores. Scanning electron microscopy was used to assess enamel surfaces for crack formation before bonding and after debonding. Statistical analysis included Scheirer–Ray–Hare test and Cochran–Armitage test of trend. **Results:** Adhesive precoated flash-free group demonstrated significantly higher ARI scores compared to conventional group (p < 0.001). Straight cutters produced the lowest ARI scores and highest crack formation, while Howe and Weingart pliers showed the highest ARI scores with minimal crack formation. Conventional brackets exhibited significantly more enamel cracks (45%) compared to APC-FF brackets (20%) (p = 0.017). Significant inverse relationship was found between ARI scores and crack formation (p < 0.001). **Conclusion:** The resultant ARI after debonding serves as a reliable predictor of potential enamel microcrack formation. APC-FF brackets demonstrated superior enamel preservation compared to conventional brackets. Among debonding techniques, Howe and Weingart pliers proved most favorable, while SCs showed highest risk of enamel damage. **Clinical significance:** Our findings posit that ARI can be a reliable predictor of enamel microcrack formation. Our findings also highlight the importance of selecting appropriate debonding methods and brackets to potentially minimize enamel harm.

ORIGINAL RESEARCH

Ahmed Khalaf Ahmed Mubarak, Mohammed Moustafa Shalaby, Ahmed Mohammed Bakry

Assessment of Imaging Accuracy Using Different Intraoral Scanner Streaming Modes: An In Vitro Study

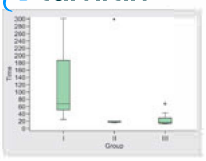
[Year:2025] [Month:April] [Volume:26] [Number:4] [Pages:6] [Pages No:397 - 402]

Keywords: Digital impression, Scanning accuracy, Scanners streaming modes, Total occlusal convergence

DOI: 10.5005/jp-journals-10024-3863 | Open Access | How to cite |

Abstract

Aim: This *in vitro* study evaluated the accuracy of two intraoral scanners with different streaming modes (CEREC Omnicam, Dentsply-Sirona, USA; video mode) and (Shining 3D, Aoralscan, China; image mode). **Materials and methods:** Three sets of acrylic maxillary typodont were uniformly reduced with known axial wall taper of 10°, 15° and 20°, respectively, using a computer numerical control (CNC) milling machine. Then, abutments were randomly divided into 3 groups: (1) Single abutments; (2) successive abutments; (3) and simple bridge-like abutments. Such abutments were scanned with three scanners: (1) Desktop scanner (InEos X5) that serve as a reference; (2) experimental intraoral scanners (CEREC Omnicam and Shining 3D). The analysis of these scans has been carried out using Geomagic Control X software to assess both IOSs trueness and precision. Each experimental model (CEREC Omnicam and Shining 3D) was scanned three times for precision determination. Descriptive analysis has been carried out by one-way ANOVA and independent *t*-test to ascertain any significant difference between the two comparing scanners. **Results:** Regarding trueness, CEREC Omnicam has significantly better trueness (0.0554 ± 0.0111 mm) than Shining 3D IOS (0.0737 ± 0.0380 mm). Meanwhile, the variance in axial wall taper demonstrated little significant variation in all groups (single, successive, and bridge-like). The significant difference is associated with shallow axial wall taper (10° taper). On the contrary, both 15° and 20° axial wall taper/total occlusal convergence (TOC) revealed no significant difference. However, no significance was revealed in regard to precision. **Conclusions:** Within the limitations of this study, the accuracy of the tested video and image streaming mode scanners is within the clinically acceptable range regarding different prosthetic scenarios, as well as different preparation convergences. **Clinical significance:** This study provides valuable insights into intraoral scanners' accuracy regarding their different streaming modes, various prosthetic scenarios, and total occlusal convergence (TOC) as well.



ORIGINAL RESEARCH

Merin A Johnson, Asha Joseph, Prabath Singh VP, Gayathri Usha, Krishnan Venugopal, Venkitachalam Ramanarayanan

An In Vitro Comparative Evaluation of Three Different Solvents in the Retreatment of Teeth Obturated using Bioceramic Sealer

[Year:2025] [Month:April] [Volume:26] [Number:4] [Pages:6] [Pages No:403 - 408]

Keywords: 10% citric acid, Bioceramic sealer, Chloroform, Endodontic retreatment, Solvent, Xylene

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Abstract

Aim: To evaluate the efficiency of three solvents in achieving patency in teeth obturated using bioceramic sealer (BCS) and to assess the effect of these solvents on the root canal wall dentine. **Materials and methods:** Twenty-one extracted single-canal mandibular premolars extracted for orthodontic purposes were selected for the study. The teeth were randomly divided into three groups (n = 7) based on the solvent used: Group I—Chloroform, group II—10% citric acid, and group III—xylene. After establishing the working length, the teeth were then instrumented and obturated using Gutta percha (GP) and BCS. Two weeks later, GP was removed, and apical patency was re-accessed with a 10-C file and the respective solvent. The time to gain patency through BC Sealer was recorded. Nine teeth (three per group) were irrigated with these solvents, and scanning electron microscopy was used to evaluate the effect of solvents on the dentin. The Kruskal–Wallis and Dunnett–Bonferroni tests were used for statistical analysis. **Results:** Patency was achieved for all samples, except for two in the chloroform group and one in the 10% citric acid group. The median (IQR) of time in group I was 60 (25–300) seconds, that of group II was 9 (6.25–10) seconds, and that of group III was 5.77 (3.66–34.34) seconds. The median time was higher in the chloroform group compared to the other groups. Kruskal–Wallis' test showed a significant difference between the three groups (p = 0.011). And on pairwise comparison, group I with group II (p = 0.017) and group III (p = 0.010) showed statistically significant differences. But there was no statistically significant difference between groups II and III (p = 1.000). Considering the scanning electron microscope evaluation, xylene caused a significant amount of open tubules in dentine, without any detritus and the smear layer covering the root surface when compared to 10% citric acid and chloroform. **Conclusion:** Xylene and 10% citric acid were more efficient than chloroform for dissolving the BCS. Statistically, there is no significant difference between xylene and 10% citric acid in achieving patency. Group III, in which xylene was used as the GP solvent, showed a significant amount of erosion compared to the other two groups. **Clinical significance:** Although BC sealer offers many advantages, the retreatment of teeth obturated with these sealers is often challenging due to its hygroscopic expansion, hard setting, and adhesion with root canal wall dentine. Solvent-assisted retreatment is less invasive, more convenient, and less time-consuming when compared with mechanical methods. Nevertheless, there is currently no efficient and practical solvent for BCSs.

ORIGINAL RESEARCH

Nourhan S Ibrahim, Wael E Jamil, Nermin A Mahmoud

Evaluation of Microtensile Bond Strength to Dentin of a Self-adhesive Bulk-fill Resin Composite Restorative Material after Aging (In Vitro Study)

[Year:2025] [Month:April] [Volume:26] [Number:4] [Pages:9] [Pages No:409 - 417]

Keywords: Aging, In vitro study, Microtensile bond strength, Self-adhesive composite

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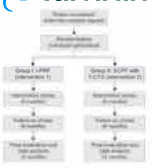
Abstract

Aim and background: Self-adhesive composites were introduced to enhance clinical and mechanical characteristics of composite restorations as they contain self-etching monomers allowing their application to tooth without additional treatment. **Aim:** To evaluate microtensile bond strength of Surefil one self-adhesive composite with and without adhesive application and Filtek One bulk-fill composite after different aging periods. **Materials and methods:** The occlusal enamel and superficial dentin of 90 sound human third molars were cut using water-cooled diamond saw. A total of 60 molars were restored using Surefil one composite, where half were applied without adhesive and the other half with adhesive. The remaining 30 molars were restored with Filtek One composite. All restored molars were exposed to thermocycling and load cycling, where each group was divided into three subgroups according to aging period (24 hours to 3–6 months). Microtensile bond strength was assessed with universal testing machine after sectioning each molar into beams with cross-sectional area 1.0 ± 0.1 mm^2. Fractured specimens were analyzed using stereomicroscope at 25× magnification to determine the failure mode. Data were analyzed using Kolmogorov–Smirnov, Shapiro–Wilk, and Pearson's Chi-square tests. **Results:** Surefil one composite without adhesive showed significantly lower bond strength than Surefil one with adhesive and Filtek one composites. Surefil one composite without adhesive showed 100% adhesive failure. Adhesive and mixed failures were noticed in both composites with adhesive application with highest percentage for adhesive failure after aging. **Conclusions:** Self-adhesive composites microtensile bond strength is enhanced with adhesive application. Conventional composite has superior microtensile bond strength than self-adhesive composite with or without adhesive application. Aging has detrimental impact on microtensile bond strength regardless of composition of composite resin. **Clinical significance:** Using adhesive with self-adhesive composites improves their bond strength. Long-term clinical results of composite are impaired by aging. More research is required for strong evidence on clinical use of self-adhesive composites.



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ORIGINAL RESEARCH

Sanehi Punse, Prasad Dhadse, Shrishti Salian, Ruchita Patil, Pavan Bajaj

Comparison of Injectable Platelet-rich Fibrin and Semilunar Flap with Tuberosity Graft for Gingival Black Triangles: A Randomized Clinical Trial

[Year:2025] [Month:April] [Volume:26] [Number:4] [Pages:8] [Pages No:418 - 425]

Keywords: Contact point, Gingival black triangles, Injectable-platelet-rich fibrin, Semilunar coronally positioned flap, Tuberosity connective tissue graft

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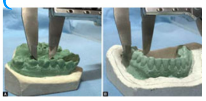
Abstract

Aim: To evaluate and compare the clinical efficacy of injectable platelet-rich fibrin (i-PRF) and semilunar coronally positioned flap (SCPF) with tuberosity connective tissue graft (T-CTG) in the management of gingival black triangles (GBTs). **Materials and methods:** Twenty patients who presented with class I and class II gingival black triangles (GBTs) were split into two groups for a randomized clinical trial: SCPF with TCTG and i-PRF. At baseline, 3, and 6 months after treatment, clinical measures such as papillary height, black triangle decrease, and periodontal pocket depth were measured. **Results:** Both groups demonstrated significant improvements in clinical parameters and patient satisfaction. The i-PRF group exhibited faster healing and greater patient comfort, while the SCPF with TCTG group achieved superior aesthetic outcomes with greater black triangle closure. However, the SCPF group experienced more postoperative discomfort and required longer healing times. **Conclusion:** The i-PRF and SCPF with TCTG are effective in managing GBTs, with each having distinct advantages. i-PRF offers a minimally invasive option with enhanced healing, whereas SCPF with TCTG provides superior aesthetic results. Clinical goals and patient requirements should guide the selection of the best approach. **Clinical significance:** The study highlights two effective approaches for managing GBTs, catering to diverse clinical and patient needs. i-PRF offers a minimally invasive method with faster healing and enhanced comfort, while SCPF with graft-CTG (SCPF with T-CTG) ensures superior aesthetic outcomes, aiding personalized periodontal therapy decisions.



ORIGINAL RESEARCH

M Kodanda Ram, KT Sangeetha Nambiar, Ajeesha Feroz, Shameema Thasneem

Tooth Dimension as a Distinguishing Trait of Sexual Dimorphism: An Odontometric Study on Kannur Population

[Year:2025] [Month:April] [Volume:26] [Number:4] [Pages:6] [Pages No:426 - 431]

Keywords: Buccolingual, Forensic odontology, Forensic sciences, Mesiodistal, Odontometrics

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Abstract

Objective: Forensic odontology deals with the application of the uniqueness of human dentition in forensic scenarios. Estimating sex is the first step in forensics as the estimation of other elements follows patterns related to sex. Our objective is to examine the relationship between odontometric measurements of permanent dentition and sexual dimorphism in the Kannur population. **Materials and methods:** A sample size of 56 paired dental casts was used to conduct the study. We included subjects aged 18–25 years. From the dental cast, measurements of mesiodistal (ML) and buccolingual (BL) distances from each tooth were taken using a digital vernier caliper. Results were tabulated and statistically analyzed. **Results:** Concerning the BL dimension, statistically significant differences were noted between the sexes. Mesiodistal dimension analysis showed no statistically significant difference. But all teeth measured were larger in males than in females. **Conclusion:** In our study done in the Kannur population, the BL dimension was seen to be statistically more significant than the MD dimension among males and females. Teeth can be a savior in mass disasters to easily recognize the recovered bodies. Further study has to be planned with a more diverse sample which can represent the Malabar population and a regression formula can be derived which can be used by forensics experts.

ORIGINAL RESEARCH

Lactobacillus Reuteri Probiotic Consumption Reduced Various Virulence Gene Expression in Dental Plaque of Fixed Orthodontic Subjects

Joko Kusnoto[1], Siti Sara Safirah[2], Litayana Ria Anggriani Sitorus[3], Winnie Valentini[4], Armelia Sari Widyarman[5]

Received on: 11 April 2025; *Accepted on*: 15 May 2025; *Published on*: 18 June 2025

Abstract

Aims: The aim of this study was to determine the effect of consuming lozenges containing *Lactobacillus reuteri* probiotic Prodentis lozenges on the expression of *BCR1*, *ACE2*, *fadA*, *aid1*, *gelE*, and *atlA* genes in dental biofilms of subjects using fixed orthodontic appliances.

Materials and methods: Plaque samples ($n = 20$) obtained from a previous study were used in this research. Each subject consumed *L. reuteri* probiotic lozenges (2×10^8 CFU/mL) each day for 2 weeks. RNA was extracted from the samples and synthesized into cDNA. The expression of the gene transcription factors *BCR1*, *ACE2*, *fadA*, *aid1*, *gelE*, and *atlA* genes in biofilms of subjects who used fixed orthodontic appliances was detected using real-time quantitative polymerase chain reaction (RT-qPCR).

Results: The expression of *BCR1*, *ACE2*, *fadA*, *aid1*, *gelE*, and *atlA* genes were decreased after consuming the *L. reuteri* probiotic lozenges for 2 weeks ($p < 0.05$).

Conclusion: Consuming *L. reuteri* probiotic lozenges would affect the expression of *BCR1*, *ACE2*, *fadA*, *aid1*, *gelE*, and *atlA* in plaque from patients using fixed orthodontic appliances. By reducing the expression of the virulence genes, bacterial number would be reduced and biofilm production can also be reduced.

Clinical significance: Consumption of probiotic lozenges were confirmed to reduce bacterial and fungal biofilm, as proven by the reduction of virulence gene expression. Routine consumption of probiotic lozenges can help reduce potential bacterial infection and increase the oral health of patients using fixed orthodontic appliances.

Keywords: Biofilm, Gene expression, *Lactobacillus reuteri*, Orthodontic, Probiotic.

The Journal of Contemporary Dental Practice (2025): 10.5005/jp-journals-10024-3857

Introduction

Orthodontic treatment is common today in the community. Among adults and children, orthodontic treatment may be undertaken for dental care or esthetic reasons.[1] Orthodontic treatment using fixed appliances aims to ensure proper occlusion and esthetic function, with appropriate tooth movement. Fixed orthodontic treatment can lead to changes in the oral environment and oral flora composition and an increase in the amount of plaque due to difficulty in maintaining oral hygiene.[2–5] In addition, excess composite around the base of the bracket that used in orthodontic treatment is an important factor that can cause plaque accumulation due to the presence of rough surfaces and cracks on the enamel composite surfaces.[3–5] Biofilm accumulation on teeth and soft tissues in the oral cavity can lead to caries, gingivitis, and periodontitis.[6,7]

Fusobacterium nucleatum is a dominant bacterial species that plays an important role in the formation of dental biofilms and periodontal tissue disease. In the formation of biofilms, *F. nucleatum* being a "bridging" or "linking" organism between initial bacterial colonization and final bacterial colonization, which are unable to bind to each other directly. *F. nucleatum* can also co-aggregate with various microbial species in the oral cavity.[8–10] *F. nucleatum* encodes several adhesion genes involved in interspecies interactions, including fusobacterium adhesion A (*fadA*), fusobacterial outer membrane protein A (*fomA*), *radD* (an arginine-inhibitable adhesin), and adherence inducing determinant gene 1 (*aid1*).[8,11–14] Fusobacterium adhesin A (FadA) is known to be involved in *F. nucleatum* invasion and adhesion to host cells and is highly conserved among oral Fusobacterium species.[8,15,16] FadA has been identified has a major virulence factor in *F. nucleatum* in interspecies interactions with *Streptococcus* mediated by *radD*, as it increases the binding specificity of *F. nucleatum* to other microbial species.[14] The arginine-inhibitable adhesion *radD* is required by *F. nucleatum* for co-adherence with various species of Gram-positive bacteria, such as streptococci (early colonizers), and fungal species, such as *Candida*.[11,12,17]

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Conflict of interest: None

Enterococcus faecalis (*E. faecalis*) is associated with chronic periodontitis and chronic apical periodontitis in failed root canal treatment.[18] *E. faecalis* is a Gram-positive aerobic bacterium. The severity of *E. faecalis* infection depends on the immune response and virulence factors, which can exacerbate infection and play a role in increasing biofilm formation.[19] There are several genes associated with *E. faecalis* biofilm formation, including gelatinase (*gelE*) and autolysin (*atlA*).[20] GelE in *E. faecalis* plaque or saliva isolates showed resistance to antibiotics and high biofilm formation ability.[21] atlA is the main peptidoglycan hydrolase or autolysin of *E. faecalis*.[22] AtlA plays a role in the biofilm maturation stage during which extracellular DNA (eDNA) is released and contributes to biofilm attachment and stability.[23,24]

An increase in the number of colonies of microorganisms also increased, one of which was *Candida albicans*, which causes infections of oral mucosa.[25,26] *C. albicans* has a protein in the form of an adhesive that mediates other microorganisms to adhere to abiotic and host surfaces to form biofilms.[27] Several *C. albicans* gene transcription factors, including biofilm and cell wall regulator 1 (*BCR1*) and angiotensin converting enzyme 2 (*ACE2*), play a role in the formation of biofilms. BCR1 acts as a major regulator of *C. albicans* biofilm formation.[28] The ACE2 transcription factor plays a role in fungal adherence, biofilm formation, and hyphal morphogenesis. In addition, ACE2 plays a role in regulating the expression of genes involved in cell wall separation and metabolism.[29] As shown in previous research, ACE2 is required for filamentation, and it can increase the number of pseudohyphae cells at the time of biofilm formation.[30]

Biofilm formation plays a role in increasing antibiotic resistance in bacterial cells. Therefore, an effective therapy is needed to prevent biofilm formation. The use of probiotics has been suggested as a promising approach to prevent and treat microbial diseases and biofilm activity in the oral cavity.[31,32] Several studies have proven that the use of probiotics has oral cavity health benefits, such as preventing caries and periodontal disease.[32,33] One commercial probiotic proven to be beneficial for oral health is *Lactobacillus reuteri*. The antimicrobial activity of *L. reuteri* inhibits colonization by pathogenic microbes and interacts the inhibition directly with host cells.[34] *L. reuteri* also inhibits the growth of *C. albicans* and *E. faecalis* biofilms.[35,36] However, no studies have investigated the effect of the probiotic *L. reuteri* on the expression of *BCR1*, *ACE2*, *fadA, aid1, gelE*, and *atlA* genes in dental plaque biofilms found in patient's oral environment with fixed orthodontic appliances. By analyzing the virulence genes found in several pathogenic bacteria, we can hypothesize that the downregulation of these genes will lead to less biofilm production and healthier oral health conditions during the duration of fixed orthodontic appliances usage. Thus, to bridge the knowledge gap, the aim of this study was to determine the effect of consuming lozenges containing the probiotic *L. reuteri* on the expression of *BCR1*, *ACE2*, *fadA*, *aid1*, *gelE*, and *atlA* genes in biofilms from subjects using fixed orthodontic appliances.

Materials and Methods

Sample Collection

Subjects undergoing fixed orthodontic therapy were enrolled in this open-label prospective, clinical trial. Ethical approval for the study was granted by the Institutional Review Board of the Faculty of Dentistry, Universitas Indonesia (approval number: 100,701,020). The inclusion criteria specified participants aged 18 years or older who had been undergoing orthodontic treatment with fixed appliances for a minimum duration of 1 year and had not consumed probiotics or antibiotics in the preceding 3 months. The fixed orthodontic appliances used by the subjects are conventional metal braces fixed appliance. Exclusion criteria encompassed individuals with systemic conditions such as hypertension or diabetes, those on systemic medications including antihypertensives, analgesics, hormonal therapies, sedatives, or anti-seizure medications, as well as individuals presenting with severe periodontal disease or known allergies to probiotics.

The study utilized *L. reuteri* Prodentis lozenges, obtained from BioGaia (Stockholm, Sweden). Each lozenge (800 mg) contained a minimum of 2×10^8 live *Limosilactobacillus reuteri* Prodentis (previously classified as *L. reuteri*). This food supplement, specifically formulated for oral health, included a proprietary combination of the *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 strains, which are recognized for their potential to support and maintain oral health.

To standardize oral hygiene practices, all participants were provided with a standardized toothbrush and toothpaste for use throughout the study period. Participants received oral hygiene instructions and were instructed to brush their teeth twice daily. Each participant was administered one probiotic lozenge containing *Limosilactobacillus reuteri* daily for 14 days, to be taken once per day after morning toothbrushing and prior to breakfast. Plaque samples were collected from participants on the day of enrolment, prior to the initiation of probiotic lozenge consumption, and again on the 14th day following the completion of the probiotic regimen.

Before sample collection, mandatory rapid antigen test for SARS-CoV-2 detection had to be taken by the subjects in response to the COVID-19 pandemic (as of 30 September 2020 when collecting the samples) and the test result had to be negative for the subject partaking in the study. In brief, the participants were instructed not to eat or drink anything 2 hours prior to collection of sample. Samples were collected using sterile cotton buds and swabbed from buccal/mesial/distal/lingual/occlusal surfaces of the index teeth of the subjects. The plaque samples were stored in sterile falcon tubes with 5 mL of phosphate-buffered saline (PBS).

Furthermore, clinical data, including the Oral Hygiene Index-Simplified score and the Papilla Bleeding Index, were recorded at each visit. Overall, 20 subjects had enrolled and fulfilled all requirements and inclusion criteria, as 20 subjects were proved to be sufficient to provide valid data as a pilot study.[37,38] Plaque samples from the subjects were continued unto the following downstream analysis.

RNA Extraction, cDNA Synthesis, and Quantification

RNA from the sample was extracted using TRIzol reagent methodology as instructed by the manufacturer (Thermo Fisher, Waltham, MA). The extracted RNA was synthesized into cDNA using ReverTra Ace™ qPCR RT Master Mix with gDNA Remover (Toyobo, Japan). The mixture for cDNA synthesis is described in Table 1. The cDNA was then quantified using an Invitrogen™ Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA). The cDNA was stored at –20°C for storage of directly used for downstream analysis.

qPCR Analysis

Amplification and detection by qPCR (Applied Biosystems, Waltham, MA) were performed. The components of the qPCR Master Mix are listed in Table 2. Using a specific kit, namely HOT FIREpol EvaGreen® qPCR Mix (Solis Biodyne, Tartu, Estonia) which was activated by

Table 1: Reagent components for DNase I reaction solution

Component	*Volume*
4 × DN Master Mix	2 mL
RNA template	0.5 pg – 0.5 mg
Nuclease-free water	X mL
Total volume	8 mL

Table 2: Components of Master Mix RT-qPCR

Component	*Volume*
5 × HOT FIREPol EvaGreen® qPCR Mix Plus	4 mL
Primer *Forward*	1 mL
Primer *Reverse*	1 mL
DNA *template*	2 mL
NFW	12 mL
Total	20 mL

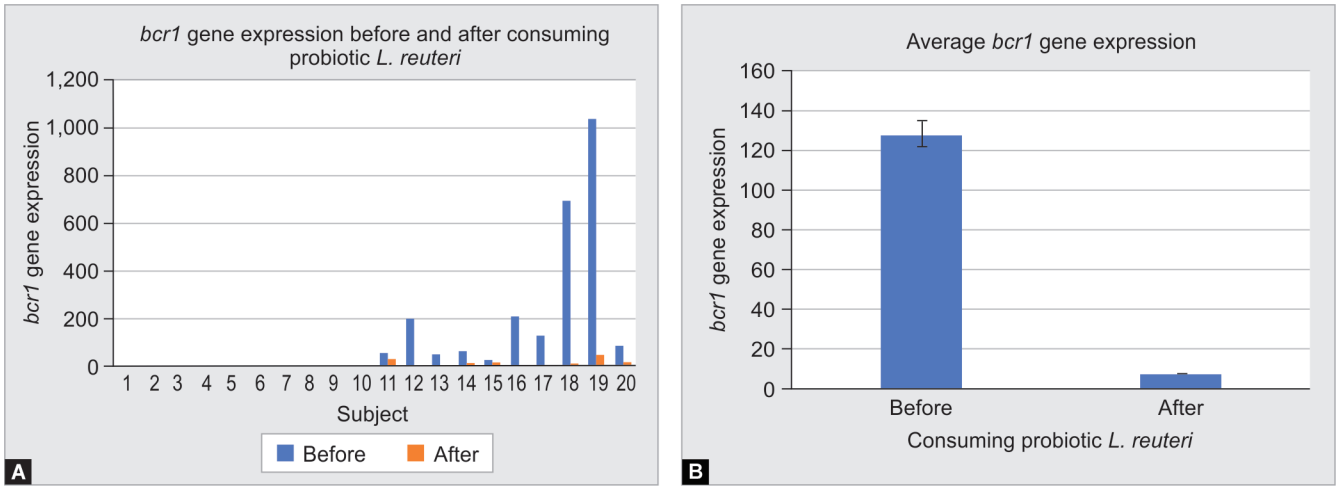
incubation at 95°C for 10 minutes. This was followed by 40 cycles of denaturation at 95°C for 10 seconds, annealing temperature at 60–65°C (Table 3), and elongation at 72°C for 20 seconds. For templates longer than 150 bp, the annealing and elongation times were extended to 30 seconds. Actin gene encoding as the housekeeping gene was used for normalization purposes. qPCR was performed on cDNA. qPCR was performed using the primers listed in Table 3.

Data Analysis and Outcome Analysis

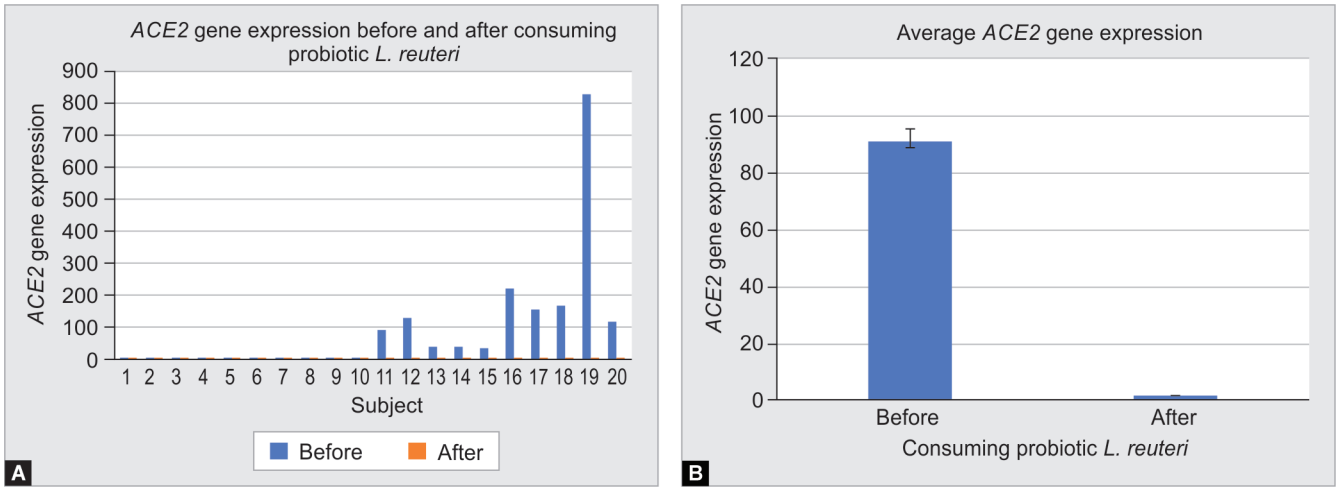
The data were analyzed using the Shapiro–Wilk normality test ($p > 0.05$). For data with a normal distribution, a paired *t*-test was applied ($p < 0.05$). The software used for the analysis is Statistical Package for the Social Sciences (SPSS) version 27 (IBM, Armonk, NY). The outcome analysis are the quantitative data presented from the RT-qPCR instrument. This data can then be correlated with previous study that also conducted such research to ensure the validity of this data.

Table 3: *Primer sequence* for RT-qPCR

Genes	*Primer sequence*	*Temperature and cycle settings*
BCR1[39]	*forward*: 5′-CTTCAGCAGCTTCATTAACACCTA-3′ *reverse*: 5′-TCTTGGATCAGGTGTACTTTTCAA-3′	Initial denaturation of 95°C for 5 minutes; 40 cycles of denaturation at 95°C for 1 minute and annealing at 58°C for 1 minute.
ACE2[40]	*forward* 5′-AGAATTGACCGTTGTCCGTGTAAG-3′ *reverse*: 5′-AATGGGTGAATAAATCCCTCCCTAA-3′	Initial denaturation 95°C for 2 minutes; 40 cycles of denaturation at 95°C for 30 seconds and annealing at 60°C for 1 minute.
Housekeeping gene *C. albicans*: *ACT1*[41]	*forward*: 5′-TTTCATCTTCTGTATCAGAGGAACTTATTT-3′ *reverse*: 5′-ATGGGATGAATCATCAAACAAGAG-3′	Initial denaturation 95°C for 10 minutes; 40 cycles of denaturing 95°C for 15 seconds and annealing 60°C for 1 minute.
fadA[15]	*forward*: 5′-CAC AAG CTG ACG CTG CTA GA- 3′ *reverse*: 5′-TTA CCA GCT CTT AAA GCT TG-3′	Initial incubation for 4 minutes at 94°C followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55.8°C for 30 seconds, and elongation at 72°C for 40 seconds and the final elongation for 6 minutes.[14]
aid1[14]	*forward:* 5′-TACAGGAG GTGCCGTAGCAG-3′ *reverse:* 5′-TTTTTGTTAATTCT CCAGCTCCA-3′	Initial incubation for 10 minutes at 95°C followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing and elongation at 60°C for 1 minute.[13]
Housekeeping gene *F. nucleatum*: *rpoB*[42]	*forward:* 5′-GGYTWYGAAGTNCGHGACGTDCA-3′ *reverse:* 5′-TGACGYTGCATGTTBGMR CCCATMA-3′	Initial incubation for 10 minutes at 95°C followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing and elongation at 60°C for 1 minute.
gelE[43]	*forward:* 5′-CGGAACATACTGCCGGTTTAGA-3′ *reverse:* 5′-TGGATTAGATGCACCCGAAAT -3′	Initial denaturation at 95°C for 3 minutes, 40 cycles of denaturation at 95°C for 5 seconds, and annealing at 60°C for 30 seconds.
atlA[44]	*forward:* 5′-AATAATCAATCAGGAACGAATACG-3′ *reverse:* 5′-GCCACACTAACACCGAAT-3′	Initial denaturation at 95°C for 2 minutes, 40 cycles of denaturation at 95°C for 15 seconds, and annealing at 60°C for 60 seconds.
Housekeeping gene *E. faecalis*: *rpoA*[44]	*forward:* 5′-GTGAAACCTGGTCGTGGCTA-3′ *reverse:* 5′-CGACGAACGGGTGTGTAGAT-3′	Initial denaturation at 95°C for 2 minutes, 40 cycles of denaturation at 95°C for 15 seconds, and annealing at 60°C for 60 seconds.



Figs 1A and B: (A) A graph showing *bcr1* gene expression in plaque samples ($N = 20$) as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges; (B) A graph showing the average of *bcr1* gene expression in plaque samples as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges



Figs 2A and B: (A) A graph showing *ACE2* gene expression in plaque samples ($N = 20$) as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges; (B) A graph showing the average of *ACE2* gene expression in plaque samples as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges

Results

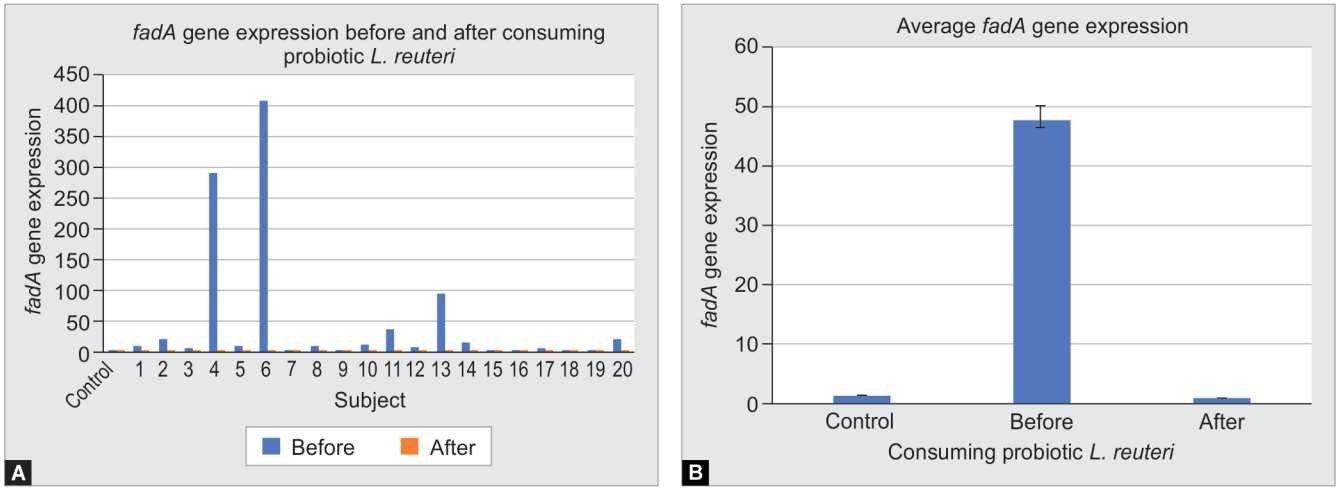
Based on the results of the qPCR test, the expression of *BCR1* (Fig. 1), *ACE2* (Fig. 2), *fadA* (Fig. 3), *aid1* (Fig. 4), *gelE* (Fig. 5), and *atlA* (Fig. 6), on average, were decreased when comparing the day-0 prior to probiotic consumption and day-14 after the subjects consumed the probiotic *L. reuteri*.

As shown by the results of the Shapiro–Wilk normality test, all the data were normally distributed ($p > 0.05$). The data were analyzed using a paired *t*-test, with a significance level of $p < 0.05$. The results of the paired *t*-test revealed a significant difference in the comparison of the *BCR1* and *ACE2* gene expression data. There was a significant difference ($p < 0.05$) in the expression of *BCR1, ACE2, fadA, aid1, gelE*, and *atlA* genes after consuming the probiotic *L. reuteri*.

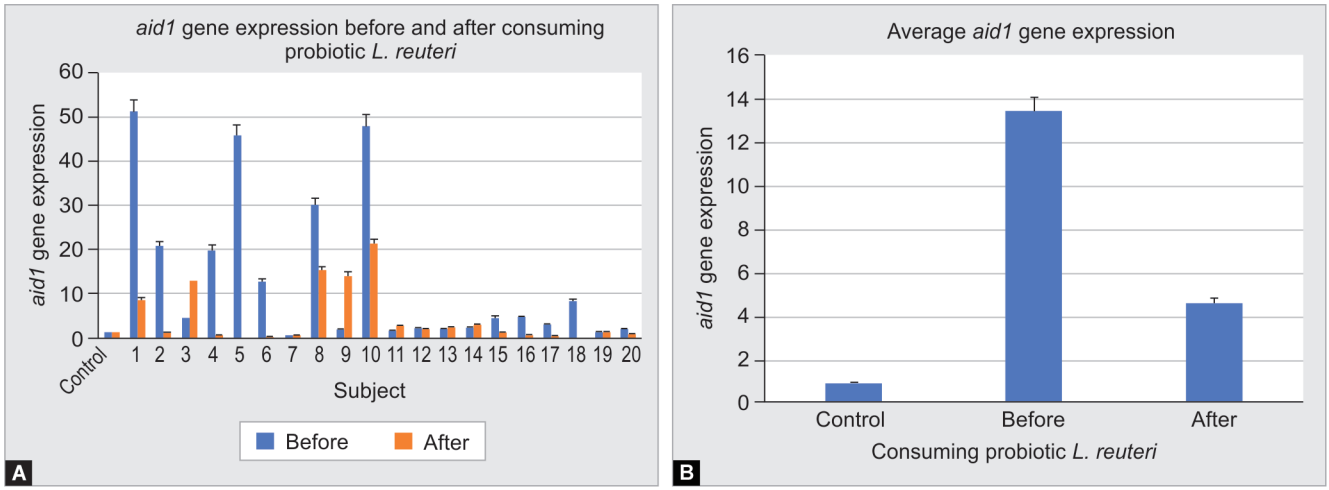
Discussion

The use of fixed orthodontic appliances often causes poor oral hygiene, thus facilitating microorganism accumulation and various pathological conditions in the oral cavity, such as fungal infections.[45] A previous study revealed an increase in *Candida* in saliva after using fixed orthodontic appliances. The authors attributed this to the design of fixed orthodontic appliances, which creates a space for the retention of food waste.[46] Thus, patients must be instructed about good oral hygiene practices after orthodontic treatment.[38] Fixed orthodontic appliances also induce changes in buffer capacity, salivary flow rates, and acidity (pH), leading to plaque accumulation and an increase in caries and periodontal disease.[47–49] Based on this information, we attempted this study to prove the benefits of consuming probiotics lozenges as a supplement for patients using fixed orthodontic appliances.

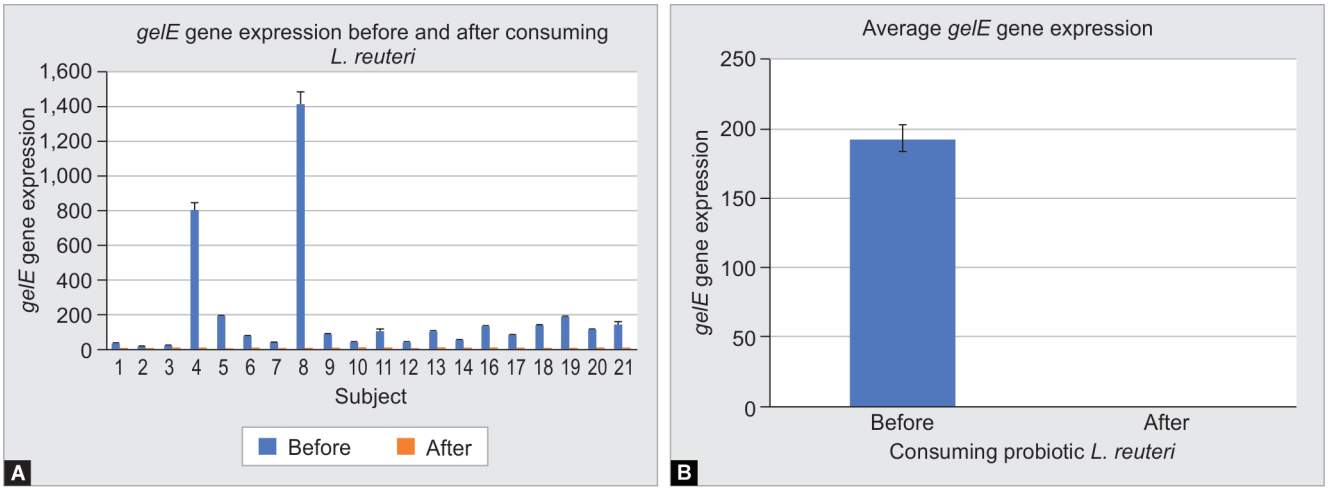
Biofilm formation is an important virulence factor of *F. nucleatum* due to its higher resistance to host defense or antibacterial agents compared to planktonic cells.[50] Several studies detected increased numbers of *Porphyromonas gingivalis, F. nucleatum, P. intermedia,* and *Tannerella forsythia* after the use of fixed orthodontic appliances.[15,51,52] They also reported that *F. nucleatum* increased



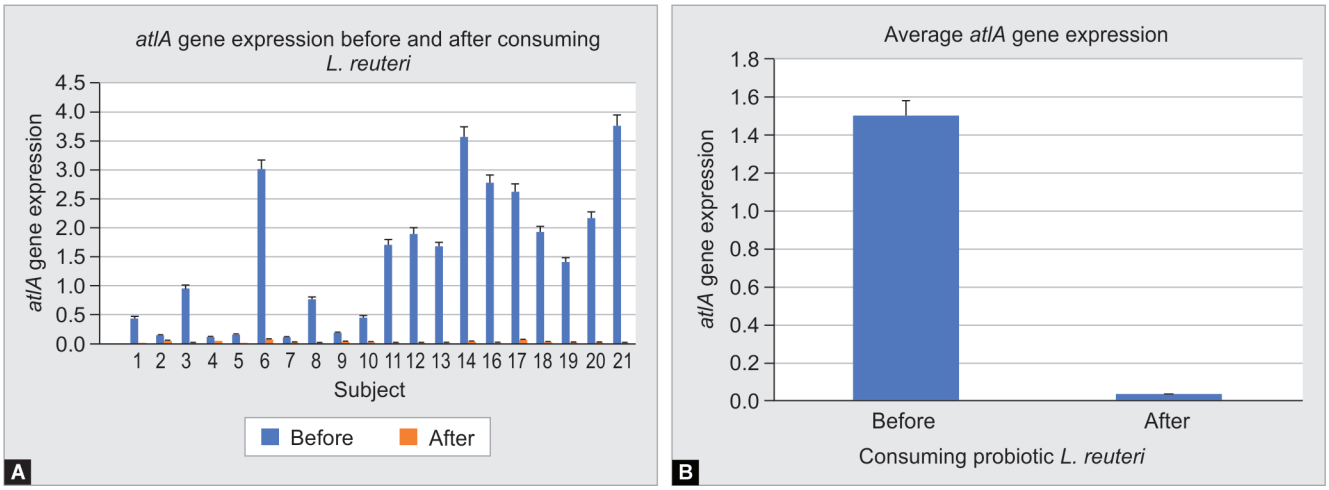
Figs 3A and B: (A) A graph showing *fadA* gene expression in plaque samples ($N = 20$) as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges; (B) A graph showing the average of *fadA* gene expression in plaque samples as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges



Figs 4A and B: (A) A graph showing *aid1* gene expression in plaque samples ($N = 20$) as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges; (B) A graph showing the average of *aid1* gene expression in plaque samples as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges



Figs 5A and B: (A) A graph showing *gelE* gene expression in plaque samples ($N = 20$) as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges; (B) A graph showing the average of *gelE* gene expression in plaque samples as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges



Figs 6A and B: (A) A graph showing *atlA* gene expression in plaque samples ($N = 20$) as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges; (B) A graph showing the average of *atlA* gene expression in plaque samples as assessed by the RT-qPCR method and the formation of *C. albicans* biofilms before and after consuming *L. reuteri* probiotic lozenges

the risk of periodontitis in orthodontic patients due to a conducive environment for anaerobic bacteria.[15,51,52] Biofilm formation is also a contributing factor to *E. faecalis* colonization and infection. Biofilms develop through various processes by which bacteria adhere to surfaces, decompose complex matrices, and develop into bacterial colonies, which adhere to surfaces.[53]

In this study, we used the qPCR method and $2^{-\Delta\Delta CT}$ formula to calculate target gene expression. As shown by the results, the probiotic *L. reuteri* affected the expression of *BCR1, ACE2, fadA*, *aid1*, *gelE*, and *atlA* genes and the formation of *C. albicans, F. nucleatum*, and *E. faecalis* biofilms. Based on the Shapiro–Wilk normality test, all the data were normally distributed, with $p > 0.05$. The paired *t*-test results revealed significant differences in the expression *of BCR1, ACE2, fadA*, *aid1*, *gelE*, and *atlA* genes (all $p < 0.05$).

Based on the results of this study, the probiotic *L. reuteri* significantly downregulated the transcription of *BCR1*, *ACE2*, *fadA*, *aid1*, *gelE*, and *atlA* genes. In gene expression, the process of translating genetic information in the form of sequence of bases of DNA or RNA into proteins.[54] Through gene expression measurement, it is possible to assess qualitatively and quantitatively the effect of a treatment, such as the administration of a drug compound.[55] Gene expression in microorganisms is involved in regulating cell-cell communication, carbohydrate metabolism, adherence, and adaptation to the surrounding environment. A decrease in the expression of specific genes can reduce microorganism colonization and microorganism numbers.[56]

BCR1, a major gene transcription factor, produces an adhesin protein, which facilitates *C. albicans* attachment to mucosal surfaces, which is a critical stage of infection.[39] Deletion of *BCR1* eliminates *C. albicans* gene function, resulting in a decrease in biofilm formation.[57] This was supported by the results of the present study, which revealed a statistically significant decrease in *BCR1* gene expression after consuming the probiotic.

The expression of *ACE2* also decreased based on the results of the statistical tests. With deletion of *ACE2*, *C. albicans* is unable to form hyphal cells, and thus biofilm formation is inhibited.[30] An earlier in vitro study showed that probiotics have antifungal effects against *C. albicans* in the oral cavity. Regular use of probiotics helped to inhibit *Candida* biofilms and reduced *Candida* colonization in the oral cavity, thereby reducing the possibility of candidiasis infection.[58]

FadA protein is the main *F. nucleatum* virulence factor and mediates microbial attachment and colonization.[8] Based on the results of this study, the probiotic *L. reuteri* appears to influence the pathogenicity of *F. nucleatum* adhesion molecules and colonization and affect biofilm formation through decreased expression of the *fadA* gene.[35] Various *F. nucleatum* adhesins mediate adhesion and aggregation and function as coaggregation intermediaries in the formation and maturation of dental biofilms.[59] The interaction of Fusobacterium with other species is largely mediated by the adhesin genes *radD* and *aid1*.[60] The *aid1* gene plays a role in interspecies interactions, colonization, and aggregation of *F. nucleatum*. In a previous study, inactivation of the *aid1* gene decreased the ability of *F. nucleatum* to aggregate, especially with *Streptococcus* spp. or *E. faecalis*.[14] As shown in earlier studies, probiotics can affect the expression of genes involved in cell adhesion, quorum sensing (QS), virulence factors, and biofilm formation.[61,62]

The *E. faecalis gelE* gene has the ability to hydrolyse gelatine, collagen, fibrin, and other peptides.[63] Gene *gelE* is a virulence factor in infection formation through bacterial attachment and biofilm formation.[22] In *E. faecalis*, biofilm formation is regulated by QS, where Fsr regulates the expression of the *gelE* gene.[64] Fsr regulates the formation of *E. faecalis* biofilms through its product *gelE* and serine proteases.[43] QS is a molecular mechanism by which bacterial cells communicate with each other via signaling molecules in biofilms. If the protease encoded by a signaling factor is decreased, communication between bacteria and biofilm formation will be disrupted.[62] GelE can also activate *atlA*, which is responsible for eDNA release at the biofilm maturation stage.[65]

AtlA is involved in the hydrolysis of peptidoglycan, which plays an important role in separating cells division after replication.[22] AtlA plays a role in the biofilm maturation stage of *E. faecalis*, during which eDNA is released and contributes to biofilm attachment and stability, biofilm defects in primary attachment, and decreased biofilm production.[20,23] This study focused on the probiotic *L. reuteri*, which has ability to secrete antimicrobial substances and compete with oral pathogens for adhesion to mucosa. In addition, *L. reuteri* can adapt and change the pH of the surrounding environment, thereby inhibiting the growth of oral pathogens.[66] The antimicrobial substances secreted by *L. reuteri* are reuterin and

reutericycline.[67] Reuterin and reutericycline are broad-spectrum antimicrobial agents that are effective against Gram-positive and -negative bacteria, fungi, and protozoa by inhibiting microbial DNA synthesis.[35,47]

Many dental and oral health care products used daily now include probiotics. The use of probiotics is increasing due to their advantages over chemical agents, namely reducing the risk of antibiotic resistance.[68] Probiotics work by modulating the immune system, producing antimicrobial substances, and inhibiting certain pathogenic organisms by interfering with adhesion, colonization, and biofilm formation. They inhibit the growth of pathogens via the production of various substances, such as lactic acid and acetic acid, which penetrate the bacterial cell membrane and lower the cytoplasmic pH of pathogenic bacteria. Hydrogen peroxide and bacteriocin can destroy the cell membrane of pathogenic bacteria and inhibit the synthesis of pathogenic DNA.[32,33,35,69] Based on the results of this study, 2 weeks of daily consumption of the probiotic *L. reuteri* affected the process of biofilm formation by downregulating the expression of *BCR1*, *ACE2, fadA, aid1, gelE,* and *atlA* genes, which function as adherent regulators and regulators of hyphae formation in biofilm formation. Many previous studies demonstrated that the addition of probiotics to dental and oral health care products. Probiotic can reduce pathogenic microorganisms in plaque samples from patients using fixed orthodontic appliances.[47,68,70–72] Therefore, it can be stated that the probiotic *L. reuteri* has good ability as an additional treatment for dental and oral health in patients using fixed orthodontic appliances.

The limitation of this study is the small sample size and the fact that the sampling had to be done during COVID-19 pandemic. Although the sample size is small, the result of this study can still provide concise and significant result. In the future, there should be larger sample size for conducting this research so the results can more accurately represent the actual population. Daily consumption of probiotic lozenges duration can also be increased for more precise and accurate results. On the other hand, consumption of probiotic lozenges research can also be conducted for other aspect of oral health diseases to promote the functionality and health-inducing aspect of probiotic lozenges, such as antiinflammation.

Conclusion

Within the limitation of this study, it can be concluded that the probiotic *L. reuteri* influences the expression of *BCR1*, *ACE2*, *fadA, aid1, gelE,* and *atlA* genes in biofilm formation. By reducing the expression of those genes, the probiotic *L. reuteri* can reduce biofilm formation, such as dental plaque, in patients using fixed orthodontic appliances.

Clinical Significance

Consumption of probiotic lozenges were confirmed to reduce bacterial and fungal biofilm, as proven by the reduction of virulence gene expression, hence, helping increasing oral health of consumer. The results of this study help clinicians provide probiotic lozenges for patients to promote and maintain their oral health.

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