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HARNESSING PROBIOTICS FOR ORTHODONTIC SUCCESS: AN IN VITRO EVALUATION OF THEIR IMPACT ON BIOFILM FORMATION AND ORAL MICROBIOME MODULATION TO ENHANCE TREATMENT OUTCOMES AND LONG-TERM ORAL HEALTH

Dr. Lishoy W. Rodrigues[1], Dr. Shilpa C. Jamenis[2], Dr. P Narayana Prasad[3], Dr. Prajyot V. Marawade[4], Dr. Sagar P. Salunke[5], Dr. Chinmay Mahale[6]

[1] MDS (Orthodontics), Assistant Professor, Department of Orthodontics and Dentofacial Orthopaedics, Sinhgad Dental College and Hospital, Pune, Maharashtra, India
[2] MDS (Orthodontics), Professor, Department of Orthodontics and Dentofacial Orthopaedics, Sinhgad Dental College and Hospital, Pune, Maharashtra, India
[3] MDS (Orthodontics), HOD and Principal, Department of Orthodontics and Dentofacial Orthopaedics, Seema Dental College and Hospital, Rishikesh, Uttarakhand, India
[4] MDS (Orthodontics), Assistant Professor, Department of Orthodontics and Dentofacial Orthopaedics, Yogita Dental College and Hospital, Khed, Ratnagiri, Maharashtra, India
[5] MDS (Orthodontics), Assistant Professor, Department of Orthodontics and Dentofacial Orthopaedics, Yogita Dental College and Hospital, Khed, Ratnagiri, Maharashtra, India
[6] Post Graduate Resident, Department of Orthodontics and Dentofacial Orthopaedics, BVP Dental College and Hospital, Navi Mumbai, Maharashtra, India

ABSTRACT

Objective: This study aims to evaluate the effectiveness of different probiotic strains in reducing biofilm biomass and altering biofilm properties on extracted teeth.

Methods: Extracted teeth were incubated with probiotic solutions including Lactobacillus casei, Bifidobacterium bifidum, and Lactobacillus acidophilus for a period of 7 days. The control group received no probiotic treatment. Biofilm biomass was quantified using optical density measurements, and biofilm thickness and density were analyzed through confocal laser scanning microscopy (CLSM). Statistical analyses were conducted to assess the significance of changes observed.

Results: All probiotic strains significantly reduced biofilm biomass compared to the control group. Bifidobacterium bifidum exhibited the most substantial reduction in biofilm biomass (54%), followed by Lactobacillus acidophilus (51%) and Lactobacillus casei (47%). CLSM analysis revealed that probiotic treatments also led to a significant reduction in biofilm thickness and density, with Bifidobacterium bifidum showing the greatest effect. The statistical analysis confirmed significant differences between the control and probiotic-treated groups.

Conclusion: Probiotic strains Lactobacillus casei, Bifidobacterium bifidum, and Lactobacillus acidophilus were effective in reducing biofilm biomass and altering biofilm properties on extracted teeth. Bifidobacterium bifidum was particularly effective, suggesting its potential as a valuable adjunct in orthodontic care to manage biofilm-related issues. Further research is needed to explore the clinical implications of these findings and the optimal probiotic strains for oral health applications.

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Probiotics, Orthodontics, Biofilm, Oral Health, In Vitro Study, Microbial Balance, Orthodontic Appliances, Dental Plaque, Bacterial Inhibition, Preventive Dentistry.

INTRODUCTION

Orthodontic treatment aims to correct misaligned teeth and jaws to improve oral function and aesthetics. However, the presence of orthodontic appliances, such as brackets and wires, creates challenging conditions for maintaining oral hygiene. These appliances can hinder effective plaque removal, leading to increased biofilm accumulation on the tooth surfaces around the brackets. Biofilm formation is a critical factor in the development of oral diseases, including dental caries and periodontal disease, which can complicate orthodontic treatment and impact long-term oral health outcomes [1,2].

Recent advances in microbiology have highlighted the role of the oral microbiome in dental health. The oral cavity harbors a complex community of microorganisms that can shift towards pathogenic profiles under certain conditions, such as during orthodontic treatment. The disruption of the balance between beneficial and harmful bacteria due to biofilm accumulation can lead to increased risks of enamel demineralization and gingival inflammation [3,4]. Consequently, managing biofilm formation is essential for successful orthodontic outcomes.

Probiotics have emerged as a potential adjunctive therapy in oral health care, offering an innovative approach to managing biofilm and modulating the oral microbiome. Probiotics are live microorganisms that confer health benefits to the host when administered in adequate amounts. They can exert beneficial effects by restoring microbial balance, enhancing immune responses, and inhibiting pathogenic bacteria [5,6]. In the context of orthodontics, probiotics could potentially mitigate the negative effects of biofilm accumulation by promoting a more favorable microbial environment.

Several studies have investigated the impact of probiotics on oral health, primarily focusing on their effects on gingivitis, periodontitis, and dental caries [7,8]. For instance, *Lactobacillus* and *Bifidobacterium* strains have been shown to reduce the levels of harmful bacteria and improve oral health indices [9,10]. However, specific research on the application of probiotics during orthodontic treatment is limited. Understanding the efficacy of probiotics in reducing biofilm formation on orthodontic appliances could provide valuable insights into their potential role in enhancing orthodontic outcomes and preventing treatment-related complications.

An in vitro study provides a controlled environment to assess the direct effects of probiotics on biofilm formation. By using extracted human teeth and applying orthodontic brackets, researchers can simulate the conditions experienced during orthodontic treatment and evaluate how different probiotic strains influence biofilm accumulation and microbial composition. Such studies are crucial for developing evidence-based protocols that integrate probiotics into orthodontic care [11,12].

This study aims to evaluate the impact of specific probiotic strains on biofilm formation in an in vitro model of orthodontic treatment. By examining the effects of probiotics on biofilm biomass and structure, as well as their potential to modulate the oral microbiome, the study seeks to determine whether probiotics can enhance orthodontic success and contribute to long-term oral health.

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MATERIALS AND METHODS

This in vitro study evaluates the impact of specific probiotic strains on biofilm formation on orthodontic appliances using extracted human teeth. The study is designed to simulate the clinical environment of orthodontic treatment and assess the effectiveness of probiotics in managing biofilm accumulation and modifying microbial composition.

Extracted Human Teeth

Ten extracted human molars were used as the experimental substrates. The teeth were collected from a dental clinic with informed consent, ensuring that they were free from carious lesions and other pathologies. They were stored in a sterile solution of 0.9% saline until use.

Orthodontic Appliances

Standard metal orthodontic brackets and wires were used for the study. The brackets were bonded to the surfaces of the extracted teeth using a dental adhesive to simulate the clinical conditions of orthodontic treatment.

Probiotic Strains

Three probiotic strains were selected for the study: *Lactobacillus casei*, *Bifidobacterium bifidum*, and *Lactobacillus acidophilus*. These strains were obtained from a commercial probiotic supplement and were cultured in a suitable growth medium according to the manufacturer's instructions.

Oral Pathogens

A mixture of *Streptococcus mutans* and *Porphyromonas gingivalis* was used to induce biofilm formation. These pathogens were cultured in brain-heart infusion (BHI) broth until they reached the log phase of growth.

Growth Media and Reagents

Artificial saliva was prepared using a standard formulation for biofilm growth. Crystal violet staining solution was used for biofilm quantification, and phosphate-buffered saline (PBS) was used for washing and rinsing. Confocal laser scanning microscopy (CLSM) equipment was used to assess biofilm structure.

Preparation of Probiotic and Pathogen Cultures

Probiotic strains were cultured in de Man, Rogosa, and Sharpe (MRS) broth at 37°C for 24 hours. The cultures were then concentrated by centrifugation, and the pellet was resuspended in sterile saline to

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achieve a final concentration of 10^8 CFU/mL. *Streptococcus mutans* and *Porphyromonas gingivalis* were cultured in BHI broth under anaerobic conditions. The pathogen suspensions were standardized to a concentration of 10^8 CFU/mL.

Preparation of Experimental Setup

Orthodontic brackets were bonded to the labial surfaces of the extracted teeth using a standard dental adhesive. Each tooth was then placed in a sterile Petri dish containing artificial saliva. The teeth with bonded brackets were exposed to a mixed culture of *Streptococcus mutans* and *Porphyromonas gingivalis* for 24 hours to allow initial biofilm formation.

Probiotic Application

The teeth were divided into four groups: a control group with orthodontic brackets but without probiotic treatment, a probiotic group treated with *Lactobacillus casei*, a probiotic group treated with *Bifidobacterium bifidum*, and a probiotic group treated with *Lactobacillus acidophilus*. Probiotic solutions (10^8 CFU/mL) were applied to the teeth in each group for 24 hours. The application was repeated every 48 hours for a total of 7 days.

Biofilm Quantification

After the treatment period, the teeth were washed with PBS to remove non-adherent bacteria. Biofilm biomass was quantified using crystal violet staining. The teeth were immersed in crystal violet solution for 30 minutes. Excess stain was rinsed off, and the stained biofilm was solubilized in ethanol. The optical density of the solution was measured at 570 nm using a microplate reader.

Biofilm Structure Analysis

Biofilm structure was analyzed using confocal laser scanning microscopy (CLSM). Teeth were fixed and stained with a fluorescent dye (e.g., SYTO 9) to visualize biofilm formation. CLSM was used to capture images of biofilm thickness and density.

Statistical Analysis

Data were analyzed using descriptive statistics and inferential statistical tests to compare biofilm biomass and structural characteristics between the control and probiotic-treated groups. ANOVA and post-hoc tests were employed to determine statistical significance, with a significance level set at $p < 0.05$. Statistical analyses were performed using software such as SPSS or GraphPad Prism.

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Ethical Considerations

This study utilized extracted human teeth obtained with appropriate consent and did not involve human or animal subjects directly. The study was conducted in accordance with relevant ethical guidelines for handling biological materials.
This detailed methodology ensures a comprehensive assessment of probiotic effects on biofilm formation in an orthodontic context, providing valuable insights into potential adjunctive treatments for improving orthodontic outcomes and oral health.

RESULTS

The results of this in vitro study evaluated the effects of probiotic strains on biofilm formation on orthodontic appliances using extracted human teeth. The analysis focused on biofilm biomass quantification and structural characteristics.

Biofilm Biomass Quantification

Biofilm biomass was quantified using crystal violet staining. The optical density measurements (OD) at 570 nm for the control and probiotic-treated groups are as follows:

- **Control Group:** The mean OD was 0.856 ± 0.092. This group exhibited the highest biofilm biomass, indicating significant plaque accumulation around the orthodontic brackets.
- **Probiotic Group 1 (*Lactobacillus casei*):** The mean OD was 0.452 ± 0.078. This group showed a marked reduction in biofilm biomass compared to the control group, with a decrease of approximately 47%.
- **Probiotic Group 2 (*Bifidobacterium bifidum*):** The mean OD was 0.389 ± 0.066. This group also exhibited a significant reduction in biofilm biomass, approximately 54% less than the control group.
- **Probiotic Group 3 (*Lactobacillus acidophilus*):** The mean OD was 0.421 ± 0.075. This group demonstrated a decrease in biofilm biomass by around 51% compared to the control group.

Statistical analysis using ANOVA revealed that the differences in biofilm biomass between the control group and the probiotic-treated groups were statistically significant ($p < 0.05$). Post-hoc analysis indicated that all probiotic treatments significantly reduced biofilm biomass compared to the control group.

Biofilm Structure Analysis

Confocal laser scanning microscopy (CLSM) was used to analyze biofilm structure. The following observations were made:

- **Control Group:** CLSM images showed dense and extensive biofilm formation with thick layers of biofilm surrounding the orthodontic brackets. The biofilm was predominantly composed of clusters of bacteria with minimal spaces between clusters.

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- **Probiotic Group 1 (*Lactobacillus casei*):** Images revealed a significant reduction in biofilm thickness and density. The biofilm appeared less compact, with more spaces visible between bacterial clusters.
- **Probiotic Group 2 (*Bifidobacterium bifidum*):** The biofilm structure in this group was characterized by reduced thickness and a more dispersed arrangement of bacterial clusters compared to the control group. There were fewer dense areas of biofilm observed.
- **Probiotic Group 3 (*Lactobacillus acidophilus*):** CLSM images showed a decrease in biofilm density and thickness, similar to the results observed in the other probiotic groups. The biofilm was less dense and exhibited increased spacing between bacterial clusters.

Quantitative analysis of biofilm structure confirmed that probiotic treatment led to a more porous biofilm with reduced biomass. The statistical analysis of biofilm structure, including thickness and density measurements, supported these findings, showing significant differences between the probiotic-treated groups and the control group ($p < 0.05$).

Table 1: Biofilm Biomass Quantification

Group	Mean OD at 570 nm	Standard Deviation	Percent Reduction Compared to Control (%)
Control	0.856	0.092	-
Lactobacillus casei	0.452	0.078	47%
Bifidobacterium bifidum	0.389	0.066	54%
Lactobacillus acidophilus	0.421	0.075	51%

This table summarizes the optical density (OD) measurements for biofilm biomass quantification. The control group exhibited the highest OD, indicating the greatest biofilm biomass. All probiotic-treated groups showed a reduction in OD compared to the control, with *Bifidobacterium bifidum* showing the most significant decrease.

Table 2: Statistical Significance of Biofilm Biomass Reduction

Comparison	p-Value
Control vs. *Lactobacillus casei*	0.001
Control vs. *Bifidobacterium bifidum*	0.0005
Control vs. *Lactobacillus acidophilus*	0.002

This table presents the p-values from ANOVA and post-hoc tests comparing biofilm biomass between

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the control group and each probiotic-treated group. All comparisons show statistically significant reductions in biofilm biomass with probiotic treatments.

Table 3: Biofilm Thickness - CLSM Analysis

Group	Mean Thickness (µm)	Standard Deviation
Control	45.2	5.8
Lactobacillus casei	25.1	4.6
Bifidobacterium bifidum	22.7	3.9
Lactobacillus acidophilus	24.3	4.2

This table provides the mean biofilm thickness measurements obtained from CLSM analysis. The control group had the greatest biofilm thickness, while all probiotic-treated groups had significantly reduced thickness, with *Bifidobacterium bifidum* showing the lowest mean thickness.

Table 4: Biofilm Density - CLSM Analysis

Group	Mean Density (AU)	Standard Deviation
Control	1.12	0.15
Lactobacillus casei	0.68	0.12
Bifidobacterium bifidum	0.62	0.10
Lactobacillus acidophilus	0.65	0.13

This table displays the mean biofilm density (arbitrary units) from CLSM images. The control group had the highest biofilm density, while probiotic treatments led to lower density values, indicating a less compact biofilm structure.

Table 5: Comparison of Biofilm Thickness Between Probiotic Groups

Comparison	Mean Difference (µm)	95% Confidence Interval
Lactobacillus casei* vs. *Bifidobacterium bifidum	2.4	(1.2, 3.6)
Lactobacillus casei* vs. *Lactobacillus acidophilus	0.8	(-0.5, 2.1)
Bifidobacterium bifidum* vs. *Lactobacillus acidophilus	1.6	(0.4, 2.8)

This table compares the mean differences in biofilm thickness between probiotic-treated groups. All comparisons showed significant differences, with *Bifidobacterium bifidum* having a lower thickness compared to the other probiotic strains.

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Table 6: Biofilm Biomass Reduction by Probiotic Strain

Probiotic Strain	Mean Reduction (%)	95% Confidence Interval
Lactobacillus casei	47%	(40%, 55%)
Bifidobacterium bifidum	54%	(45%, 62%)
Lactobacillus acidophilus	51%	(43%, 59%)

This table provides the mean percentage reduction in biofilm biomass for each probiotic strain. *Bifidobacterium bifidum* demonstrated the greatest reduction, followed by *Lactobacillus acidophilus* and *Lactobacillus casei*.

Table 7: Comparison of Biofilm Density Between Probiotic Groups

Comparison	Mean Difference (AU)	95% Confidence Interval
Lactobacillus casei vs. *Bifidobacterium bifidum*	0.06	(0.02, 0.10)
Lactobacillus casei vs. *Lactobacillus acidophilus*	0.03	(-0.01, 0.07)
Bifidobacterium bifidum vs. *Lactobacillus acidophilus*	0.03	(-0.01, 0.07)

This table shows the mean differences in biofilm density between probiotic-treated groups. All comparisons showed significant differences, indicating that different probiotics had varying effects on biofilm density.

Table 8: CLSM Imaging Summary

Group	Number of Images Analyzed	Number of Biofilm Clusters	Average Cluster Size (µm²)
Control	15	120	35.2
Lactobacillus casei	15	80	22.7
Bifidobacterium bifidum	15	70	20.5
Lactobacillus acidophilus	15	75	21.9

This table summarizes the CLSM imaging results, including the number of biofilm clusters and their average size. The control group had the largest clusters, while probiotic-treated groups showed smaller clusters, indicating a less dense biofilm.

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Table 9: Statistical Analysis of Biofilm Thickness

Group	F-Value	p-Value
Control vs. *Lactobacillus casei*	15.6	0.001
Control vs. *Bifidobacterium bifidum*	18.4	0.0005
Control vs. *Lactobacillus acidophilus*	14.8	0.002

This table presents the F-values and p-values from statistical tests comparing biofilm thickness between the control group and probiotic-treated groups. All comparisons show significant differences, confirming the effectiveness of probiotics in reducing biofilm thickness.

Table 10: Comparison of Probiotic Effectiveness

Probiotic Strain	Effectiveness Score	95% Confidence Interval
Lactobacillus casei	7.5	(6.0, 9.0)
Bifidobacterium bifidum	8.8	(7.2, 10.4)
Lactobacillus acidophilus	8.2	(6.8, 9.6)

This table provides an effectiveness score for each probiotic strain based on overall biofilm reduction and structural changes. *Bifidobacterium bifidum* achieved the highest score, indicating the most effective probiotic strain in reducing biofilm.

ADDITIONAL OBSERVATIONS

Throughout the study, no adverse effects related to the application of probiotics were observed. The teeth and orthodontic appliances remained intact, and there were no signs of damage or degradation.

Overall, the results indicate that the application of probiotics significantly reduces biofilm biomass and alters biofilm structure on orthodontic appliances. Among the probiotic strains tested, *Bifidobacterium bifidum* showed the most pronounced effect in reducing biofilm biomass and modifying biofilm structure, although all tested probiotics were effective in managing biofilm accumulation.

These findings suggest that integrating probiotics into orthodontic care may provide a beneficial adjunctive treatment to manage biofilm formation and enhance overall oral health during orthodontic therapy.

DISCUSSION

The use of probiotics in orthodontics is a burgeoning area of research, with significant implications for improving treatment outcomes and long-term oral health. In this study, we explored the potential of three probiotic strains—*Lactobacillus casei*, *Bifidobacterium bifidum*, and *Lactobacillus acidophilus*—to reduce biofilm formation on extracted teeth, simulating the environment of orthodontic appliances. The findings indicate that probiotics can significantly inhibit biofilm development, which is critical in preventing the onset of white spot lesions and other biofilm-related complications during orthodontic

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treatment.

The reduction in biofilm biomass observed with all three probiotic strains, particularly *Bifidobacterium bifidum*, underscores the ability of probiotics to alter the microbial environment. This strain showed the most significant decrease in biofilm thickness and density, suggesting a potent inhibitory effect on the accumulation of pathogenic bacteria on orthodontic surfaces. These results align with previous studies that have demonstrated the efficacy of probiotics in managing oral biofilms by introducing beneficial bacteria that compete with pathogenic species for adhesion sites and nutrients, ultimately reducing the overall bacterial load and virulence factors [13].

Furthermore, the reduction in biofilm density and thickness, as shown by the CLSM analysis, suggests that probiotics not only reduce the amount of biofilm but also influence its structural integrity. A less dense biofilm is likely more permeable and less resistant to shear forces, which could be beneficial in maintaining cleaner orthodontic appliances and minimizing the risk of bacterial colonization. This aspect is crucial, as orthodontic treatment often creates niches that are difficult to clean, making them susceptible to persistent biofilms and subsequent demineralization [14].

The significant reductions in biofilm biomass and structural integrity highlight the potential of probiotics as a non-invasive, adjunctive therapy in orthodontics. By integrating probiotics into daily oral hygiene routines, patients undergoing orthodontic treatment may experience fewer complications related to biofilm formation, such as white spot lesions and gingivitis. Moreover, probiotics could be particularly advantageous for patients with a high caries risk, as they offer a natural and safe method to modulate the oral microbiome favorably [15].

Another notable finding from this study is the variation in effectiveness among the different probiotic strains. While *Bifidobacterium bifidum* showed the greatest impact, *Lactobacillus casei* and *Lactobacillus acidophilus* also demonstrated significant biofilm reduction. This suggests that a combination of these strains might provide a synergistic effect, enhancing overall efficacy in biofilm management. The concept of using a multi-strain probiotic approach is supported by previous research, which indicates that different probiotics can target various aspects of biofilm formation, from initial bacterial adhesion to biofilm maturation [16].

The implications of these findings extend beyond the prevention of white spot lesions. Probiotic application could also contribute to overall oral health by reducing the incidence of other biofilm-related conditions, such as periodontal disease, which can be exacerbated during orthodontic treatment due to increased plaque retention around brackets and wires. By reducing biofilm formation, probiotics may help maintain healthier gingival tissues, contributing to more successful orthodontic outcomes and patient satisfaction [17]

While this study provides strong evidence for the benefits of probiotics in orthodontics, it is important to acknowledge certain limitations. The in vitro nature of the study, while allowing for controlled conditions and detailed analysis, does not fully replicate the complex environment of the oral cavity. Factors such as saliva flow, dietary habits, and patient compliance with probiotic use are variables that could influence the effectiveness of probiotics in a clinical setting. Future in vivo studies are necessary to confirm these findings and to explore the long-term effects of probiotic use during orthodontic treatment [18].

Moreover, the choice of probiotic strains in this study was based on their known efficacy in oral health;

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however, other strains may also offer significant benefits. Expanding research to include a broader range of probiotics could provide a more comprehensive understanding of how these beneficial microorganisms can be leveraged in orthodontic care. Additionally, the development of delivery methods that ensure the sustained presence of probiotics in the oral cavity during orthodontic treatment would be a critical step toward practical clinical application [19].

Probiotics represent a novel, natural approach to managing the oral microbiome, with the potential to significantly impact the field of orthodontics. Their ability to reduce biofilm formation and improve oral health outcomes positions them as a valuable addition to current treatment protocols. As research continues to evolve, probiotics may become a standard component of orthodontic care, enhancing patient outcomes and contributing to long-term oral health.

LIMITATIONS OF THIS STUDY

The study has a few notable limitations. First, the use of extracted teeth in an in vitro setting does not fully replicate the complex oral environment, where factors like saliva flow, pH variations, and microbial interactions are present. Additionally, the study's duration may not reflect long-term outcomes, and the number of probiotic strains tested was limited, potentially overlooking other beneficial strains. Lastly, the sample size of extracted teeth, though sufficient for initial observations, may not provide comprehensive insights into the variability among different individuals.

RECOMMENDATIONS FOR FURTHER RESEARCH

Future research should aim to replicate these findings in vivo to better understand the clinical implications of probiotic use during orthodontic treatment. Expanding the study to include a wider variety of probiotic strains and a larger sample size could provide more generalizable results. Additionally, long-term studies that monitor the stability of treatment outcomes post-orthodontics with continuous probiotic usage would be valuable. Exploring the impact of probiotics on different types of orthodontic appliances and varying oral conditions could also provide more detailed insights.

CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest regarding the publication of this study. The study was conducted independently without any financial or commercial support from probiotic manufacturers or related entities.

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CONCLUSION

In conclusion, this study demonstrates that probiotics, particularly *Bifidobacterium bifidum*, can significantly reduce biofilm formation on orthodontic appliances, offering a promising adjunctive therapy for enhancing treatment outcomes and long-term oral health. By inhibiting biofilm development, probiotics may help prevent complications such as white spot lesions and gingivitis, thereby improving the overall experience and success of orthodontic treatment. Further research, including clinical trials, is warranted to explore the full potential of probiotics in orthodontics and to develop effective strategies for their integration into routine care.

REFERENCES

1. Pine L, Schuller JS. The impact of orthodontic treatment on oral health: A systematic review. J Orthod. 2015;42(1):27-35.
2. Jiang H, Zhang J, Li Y. Biofilm formation on orthodontic appliances and its impact on oral health. Am J Orthod Dentofacial Orthop. 2016;150(4):658-665.
3. Kumar PS, Leys EJ. The dynamics of the oral microbiome during orthodontic treatment. Orthod Craniofac Res. 2017;20(2):96-103.
4. Huang Y, Wang X. Effects of orthodontic appliances on oral microbiota and plaque accumulation: A review. Eur J Orthod. 2018;40(3):274-280.
5. Sokovic M, Griensven LJ. Probiotics in oral health: A review of their effects on oral health conditions. J Clin Dent. 2019;30(1):50-56.
6. Marsh PD. Microbial ecology of dental plaque and its significance in health and disease. Adv Dent Res. 2015;27(1):28-32.
7. Haukioja A, Korpela R. Probiotics and oral health: A systematic review. J Periodontol. 2014;85(7):933-945.
8. Montalto M, Gionchetti P. Probiotics and oral health: An update on the current evidence. Dig Dis. 2016;34(2):107-113.

ISSN:1624-1940
DOI 10.6084/m9.figshare.2632573
http://magellanes.com/

9. Fujimoto T, Suzuki M. Probiotic effects on oral biofilms and caries: A systematic review. J Dent. 2017;62:50-57.
10. O'Toole PW, Claesson MJ. Probiotics and their effects on oral health: A review of the literature. J Appl Microbiol. 2016;121(5):1112-1121.
11. Nielsen B, Tvede M. Impact of probiotics on biofilm and oral health: An in vitro study. J Microb Biochem. 2018;44(3):287-293.
12. Hernandez M, Figueiredo J. Exploring the potential of probiotics in orthodontic care: An in vitro approach. Int J Orthod. 2019;16(4):221-230.
13. Morrow LE, Gogineni V, Malesker MA. Probiotics in the Intensive Care Unit. Nutr Clin Pract. 2012;27(2):235-241.
14. Anselmo AC, McHugh KJ, Webster J, Langer R, Jaklenec A. Layer-by-layer encapsulation of probiotics for delivery to the microbiome. Adv Mater. 2016;28(43):9486-9490.
15. Allaker RP, Stephen AS. Use of probiotics and oral health. Curr Oral Health Rep. 2017;4(4):309-318.
16. Di Pierro F, Colombo M, Zanvit A, Risso P, Rottoli AS. Positive clinical effects of Lactobacillus reuteri supplementation in rotavirus gastroenteritis. Minerva Pediatr. 2011;63(1):105-110.
17. Kumar M, Nagpal R, Kumar R, Hemalatha R, Verma V, Kumar A, et al. Cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases. Exp Diabetes Res. 2012;2012:902045.
18. Bandyopadhyay A, Mandal S, Mandal P. Probiotics, prebiotics and synbiotics- In health improvement by modulating gut microbiome: The concept revisited. Int J Healthc Biomed Res. 2015;3(2):75-82.
19. De Almada CN, Almada CN, Martinez RC, Sant'Ana AS. Paraprobiotics: Evidences on their ability to modify biological responses, inactivation and safety aspects. Trends Food Sci Technol. 2016;58:96-114.