A Comparison of *Lactobacillus acidophilus* Adhesion to Metal and Ceramic Brackets with Coated and Uncoated Nickel Titanium Orthodontic Archwires: An In Vitro Study

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ABSTRACT

Background and Objective: According to microbiological investigations, microorganisms, especially Lactobacillus strains, considerably increase after using fixed orthodontic appliances. One of the Lactobacilli bacteria found in the oral cavity is *Lactobacillus acidophilus*. The purpose of this study was to compare the adhesion of *Lactobacillus acidophilus* to metal and ceramic brackets with coated and uncoated nickel titanium (NiTi) orthodontic archwires.

Methods: Forty () samples were divided into four groups for this in vitro study: 10 metal brackets with coated NiTi archwire, 10 metal brackets with uncoated NiTi archwire, 10 ceramic brackets with coated NiTi archwire, and 10 ceramic brackets with uncoated NiTi archwire. Elisa Reader was used to count the number of *Lactobacillus acidophilus* attachments, and the one-way ANOVA and Tukey HSD tests were used to analyze all results.

Results: The results showed significant differences in the attachment of *Lactobacillus Acidophilus* between the ceramic bracket and coated NiTi archwire sample groups and the metal bracket and uncoated NiTi archwire sample groups (P= 0.01). The adherence of *Lactobacillus acidophilus* to the ceramic bracket and uncoated NiTi archwire group was higher than the metal bracket and coated NiTi archwire group, and the metal bracket and uncoated NiTi archwire group. The attachment of *Lactobacillus acidophilus* to the metal bracket and uncoated NiTi archwire groups was the lowest of all sample groups in this study.

Conclusion: The highest *Lactobacillus acidophilus* adherence was in the ceramic bracket with coated NiTi archwire group compared to the other three groups.

Keywords: Lactobacillus acidophilus, orthodontic bracket, orthodontic wires

Paper presentation – Thesis Results Seminar, Faculty of Dentistry, Hasanuddin University, December 16, 2022.

eISSN 2094-9278 (Online)
Published: December 13, 2024
https://doi.org/10.47895/amp.vi0.7945

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INTRODUCTION

The science and technology of orthodontic treatment are advancing daily along with the rising expectations of the public, who are aware that the function of the teeth is crucial to attractiveness.[1] Although many adult patients seek orthodontic treatment, previous studies have revealed that almost 30% of patients do not want a treatment using orthodontic appliances that are attached to the labial of the teeth.[2] These considerations contributed to advancements in dental materials research, which in turn led to the invention of aesthetic brackets and archwires, as well as the accessibility of other aesthetic tools.[3]

Patients who need orthodontic treatment are currently regarded as low-risk patients, and orthodontic treatment methods fall within the category of noninvasive treatments. The condition of oral hygiene might get worse during orthodontic treatment, especially fixed orthodontics; this is frequently attributed to the patient's difficulty in cleaning.[4] Brackets, bands, bonding materials, archwires, and other accessories make this problem worse by acting as plaque retention sites, which can result in gingivitis, enamel demineralization, white spots, and caries. It is important to prevent these risks and difficulties. The crown, pulp, root, alveolar bone, and periodontal tissue are just a few of the treatment's complications and risks that can result from orthodontic appliances.[2]

According to microbiological investigations, microorganisms, especially *Lactobacillus* and *Streptococcus* strains, considerably increase after using fixed orthodontic appliances. One of the Lactobacilli bacteria found in the oral cavity is *Lactobacillus acidophilus*. The most prevalent Lactobacillus species that cause dental caries is *Lactobacillus acidophilus*.[4] This bacterium, which is gram-positive and capable of anaerobic growth, frequently causes secondary caries lesions and has the ability to speed up the demineralization process, which is a factor in the initiation and progression of caries. The number of bacteria in the mouth cavity tends to rise as a result of orthodontic braces because they tend to produce a new surface that is suitable for plaque production.[5] Up to 50% of orthodontic patients experience enamel demineralization and periodontal disease after fixed orthodontic treatment. Within a month of starting treatment, white spot lesions around braces are a possibility. Thus, it is crucial for orthodontists to prevent microorganisms from adhering to orthodontic appliances.[6]

The usage of aesthetic brackets and archwires is growing in popularity as orthodontic treatment for adult patients is becoming more common. However, several studies have demonstrated that the use of orthodontic appliances brings about changes in the oral environment, including increased levels of *Lactobacillus acidophilus* in the saliva and biofilm. Therefore, studies evaluating the relationship of these microorganisms and various types of orthodontic appliances especially those that are frequently used should be encouraged and carried out to address these questions. For that reason, scientists are interested in analyzing the variations on how *Lactobacillus acidophilus* adheres to metal and ceramic brackets with coated and uncoated nickel titanium archwire.

MATERIALS AND METHODS

Experimental laboratory research is the methodology employed. This study was carried out at Hasanuddin University Hospital's Research Laboratory, Makassar, Indonesia. The Federer formulation was used to calculate the amount of samples in this investigation. In each group, six samples are taken based on the measurement results. The amount of sample is increased to 10 samples for each group in order to prevent loss control of the sample. The samples for this investigation were divided into four groups: a metal bracket with coated nickel titanium archwire (n=10), a metal bracket with uncoated nickel titanium archwire (n=10), a ceramic brackets with uncoated nickel titanium archwire (n=10) and a sample group of ceramic brackets with coated nickel titanium archwire (n=10). The study included 40 brackets, consisting of 20 ceramic and 20 metal premolar brackets (Protect Bracket Roth prescription slot 0.022), as well as 40 pieces of nickel titanium archwire - 20 coated nickel titanium archwire (Ortho Tech, Epoxy full coated NiTi 0.014 round) and 20 uncoated nickel titanium archwire (American Orthodontic NiTi archwire 0.014 round), each measuring 5 mm. This study's inclusion criteria are as follows: premolar brackets made of ceramic that have never been used by an orthodontic patient, a coated NiTi archwire that has never been used by an orthodontic patient, uncoated NiTi archwire that has not been used by any orthodontic patient, premolar bracket made of metal that has never been used by an orthodontic patient, ceramic and metal orthodontic appliances satisfy the ISO standards, and both coated and uncoated NiTi archwire satisfy the ISO standards. The exclusion criteria consist of: metal and ceramic premolar brackets that are defective, and both coated and uncoated NiTi archwire are defective. All of these samples complied with the inclusion and exclusion criteria. A ligature wire with three threads was used to ligate each bracket and archwire group. Prior to putting into the microplate (IWAKI microplate), the autoclave was used to sterilize each sample group to be evaluated. The microplate was filled with the sample. then added 100 ml of *Lactobacillus* A*cidophilus* MRS Broth isolate, 50 ml of sucrose, and 50 ml of artificial saliva (Figure 1). The sample-containing microplate was incubated for 24 hours at 37°C with 150 rpm shaking (Heidolph Incubator 1000). The sample was taken out of the microplate,

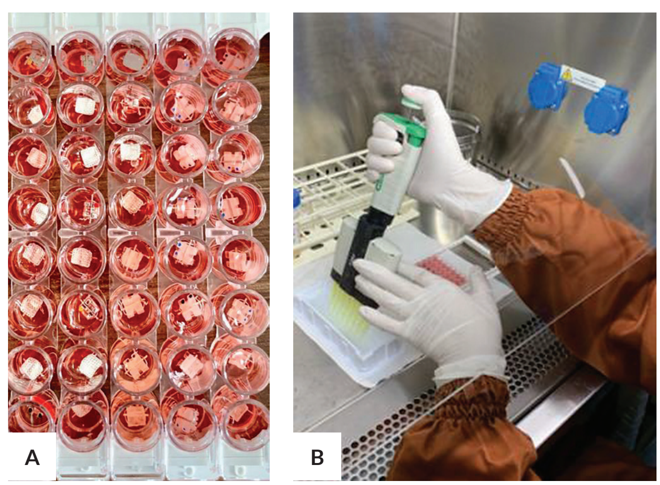

Figure 1. (A) The microplate was filled with the sample. **(B)** 100 ml of *Lactobacillus Acidophilus* MRS Broth isolate, 50 ml of sucrose, and 50 ml of artificial saliva were added.

Table 1. Number of *Lactobacillus acidophilus* Attachments to Orthodontic Appliances

	Metal bracket and coated archwire	Metal bracket and uncoated archwire	Ceramic bracket and coated archwire	Ceramic bracket and uncoated archwire	P value
	Mean ± SD				
Lactobacillus acidophilus attachments	0.49 ± 0.27	0.31 ± 0.18	0.72 ± 0.40	0.63 ± 0.24	0.015

Table 2. Comparison of the Number of *Lactobacillus acidophilus* Attachments to Orthodontic Appliances

	Sample group	Comparison	Mean	P value
Lactobacillus acidophilus attachments	Metal bracket and coated archwire	Metal bracket and uncoated archwire	0.18	0.48
	Ceramic bracket and coated archwire	Ceramic bracket and uncoated archwire	0.08	0.91
	Ceramic bracket and coated archwire	Metal bracket and uncoated archwire	0.41*	0.01
	Ceramic bracket and coated archwire	Metal bracket and coated archwire	0.23	0.30
	Ceramic bracket and uncoated archwire	Metal bracket and coated archwire	0.14	0.68
	Ceramic bracket and uncoated archwire	Metal bracket and uncoated archwire	0.33	0.07

washed once with PBS (1 tablet of pure PBS and 100μL dH_2O) for 2 minutes, then dried, followed by 30 minutes of dark incubation (Memmert Incubator) at 37°C after staining with 1% crystal violet. The microplate was then cleaned of the crystal violet, and was dried on absorbent paper. The microplate was subsequently rinsed five times in dH_2O 200 μl before being dried. The microplate was inserted into the Elisa reader (Thermo Scientific Elisa Reader) after adding 200 ml of the extraction solution (dH_2O 100 μl, alcohol 80 μl, glacial acetic acid 20 μl) and the absorbance value will be displayed along with an Optical Density value. Statistical Package for Social Science (SPSS) version 24 was used for the statistical analysis. Statistical tests include the one-way ANOVA test and the Tukey HSD test.

RESULTS

Based on Table 1, the average level of *Lactobacillus acidophilus* attachment was obtained with an Optical Density (OD) value of 0.49 ± 0.27 in the sample group made up of metal brackets and coated archwires, followed by the metal bracket and uncoated archwire group with an OD value of 0.31 ± 0.18, the ceramic bracket and coated archwire group with an OD value of 0.72 ± 0.40, and the ceramic bracket and uncoated archwire group with an OD value of 0.63 ± 0.24. The ceramic bracket and coated archwire group had the highest level of *Lactobacillus acidophilus* attachment compared to the other three groups. Among the sample groups, *Lactobacillus acidophilus* attachment to the metal bracket and uncoated archwire groups was the lowest.

Table 2 shows a significant difference in *Lactobacillus acidophilus* attachment between the ceramic bracket and coated archwire group and the metal bracket and uncoated archwire group, with a P value = 0.01. *Lactobacillus Acidophilus* attachment differed by 0.18 between the metal bracket and coated archwire and the metal bracket and uncoated archwire, and by 0.23 between the metal bracket and coated archwire and the ceramic bracket and coated archwire. The difference in the attachment of *Lactobacillus acidophilus* between the metal bracket and coated archwire groups and the ceramic bracket and uncoated archwire groups was 0.14. The difference in *Lactobacillus acidophilus* attachment between the metal bracket and uncoated archwire group and the ceramic bracket and coated archwire group was 0.41. *Lactobacillus acidophilus* attachment differed by 0.33 between the groups of metal brackets and uncoated archwires and ceramic brackets and uncoated archwires. Between the ceramic bracket and coated archwire group and the ceramic bracket and uncoated archwire group, there was a 0.08 difference in *Lactobacillus acidophilus* attachment.

DISCUSSION

Fixed orthodontic appliance components consist of brackets, bands, archwires, elastics, o rings, and power chains. Fixed orthodontic devices in the oral cavity may facilitate bacterial growth.[7] The presence of the bracket and archwire creates a barrier that prevents the brushes from effectively cleaning the teeth, which causes an excessive buildup of plaque on the bracket and archwire's surface. One of the reasons of periodontal disease and caries is plaque. Dental caries starts when there is plaque on the teeth's surface.[5] Bacteria then break down the sucrose in food scraps into lactic acid, which lowers the mouth's pH and demineralizes the enamel, starting the process that leads to dental caries. *Lactobacillus acidophilus* is one of the bacteria that causes dental caries.[8]

The results of this study showed the presence of differences in *Lactobacillus acidophilus* adherence in each sample group. According to a study by Saloom et al.,[4] orthodontic appliance materials have an impact on the adherence and growth of bacteria.[4] Lactobacillus Acidophilus adherence varied significantly between groups C (ceramic bracket and coated archwire) and B (metal bracket and uncoated archwire) with a value of P = 0.01. Group C (ceramic bracket and coated archwire) had the highest *Lactobacillus* *acidophilus* adherence compared to group B (metal bracket and uncoated archwire), This is in line with the results of an *in vivo* study on bacterial colonization with metal, ceramic and self-ligating brackets by Raju et al., which showed that plaque retention was higher in ceramic brackets tied using elastomeric rings than metal brackets tied with ligature wire.[9] This study is also in line with a study by Costa et al.[8] who evaluated the adhesion of microbes between fully coated archwires, partially coated archwires, and uncoated archwires, and found that the accumulation of microorganisms on fully coated archwires was the largest compared to other research samples.[8] Similar results were found in a study by Hasipek et al.[10] who compared the adhesion of Lactobacillus and streptococcus bacteria to coated-uncoated NiTi archwire and found that, after intraoral usage for four weeks in the same patient, Lactobacillus colonies were more numerous on Epoxy resin coated NiTi archwire than uncoated NiTi archwire.[10]

Ceramic is a biocompatible bracket material that is suitable for use because of its aesthetic value, strength, hygienic qualities, and tissue compatibility. There are three structural types of ceramic brackets on the market: zirconium, monocrystalline alumina, and polycrystalline alumina.[11] In comparison to other ceramic brackets, crystal mono alumina brackets are more durable and have smoother slot surfaces. In contrast, ceramic brackets composed of polycrystalline alumina have a rough surface, and the wings might crack under excessive torsion force. The ceramic bracket employed in this study is a polycrystalline alumina bracket, which has a rougher surface than a metal bracket and has an impact on the amount of *Lactobacillus acidophilus* adhesion.[12]

Fixed orthodontic appliance with a coated archwire that is more aesthetic but has a rougher surface, changes in mechanical qualities, corrodes more, discolors more, and can peel coating.[8] The interaction between the archwires, brackets, and ligatures, particularly in the sample group with coated archwires, may contribute to the increase in surface roughness. An epoxy-coated NiTI archwire was the particular type of coated archwire used in this study. Teflon is smoother than epoxy coating. The Teflon coating only thickens the archwire by a minimum of 0.0008 to 0.001 inches, however the epoxy coating thickens the archwire by as much as 0.002 inches, giving it a thicker coating than Teflon. The coated archwire's surface may start to peel off, increasing the surface roughness and the possibility of bacteria accumulating on the exposed surface.[13]

Orthodontic materials' uneven and rough surface textures provide an area for plaque buildup and accumulation, which can increase the amount of microorganisms in the oral cavity.[4] Microorganisms make up 70% of plaque and intercellular material makes up 30%. According to microbiological research, the quantity of bacteria, particularly Lactobacillus and Streptococcus strains, increases dramatically following the placement of fixed orthodontic appliances, disrupting the oral environment and providing an environment for disease to develop.[5] Components of an orthodontic appliance can easily become covered in dental plaque in hidden locations.[8] If a fixed orthodontist does not maintain hygiene, plaque will increase. Carbohydrates cause plaque to build, whereas both plaque and carbohydrates causes caries. *Lactobacillus acidophilus* is one of the bacteria that contributes to tooth caries.[14]

In the oral cavity, *Lactobacillus acidophilus* produces lactic acid from fermented sugars, which decreases the pH level of the plaque and can lead to demineralization on the surface of the tooth.[5] The amount of *Lactobacillus acidophilus* in saliva can be influenced by a number of factors, including carbohydrate intake. The metabolism of sucrose, which is involved in two pathogenic features, such as adhesion and acid production, is related to the cariogenic capabilities of bacteria. Because lactic acid, a more cariogenic byproduct of carbohydrate metabolism, is produced by *Lactobacillus acidophilus*, this bacterium is one of the factors contributing to dental caries in humans. Lactobacillus has aciduric properties that can tolerate acids. This situation allows these bacteria to survive in dental plaque and carious areas, and continue to damage the tooth structure.[15]

The three types of carbohydrates are monosaccharides, disaccharides, and polysaccharides. Disaccharides can be divided into three categories: lactose, maltose, and sucrose. The growth rate of organisms can be dramatically impacted by the presence of carbohydrates like sucrose.[16] The research samples in this study had sucrose added as a substrate. Sucrose was selected as the disaccharide since it is the most widely used and accessible. According to research by Srinivas et al.,[17] adding glucose or fructose to milk encouraged the growth of *Lactobacillus acidophilus* and increased the amount of acid that *Lactobacillus acidophilus* produced. The addition of sucrose in this investigation yielded results that were consistent with their findings.[17]

According to the report's results, group B's (metal bracket and uncoated archwire) level of *Lactobacillus acidophilus* adherence was the lowest of all sample groups. According to a research by Brusca et al.,[18] the composition of the bracket determines how many microbes adhere and develop. His research revealed that metal brackets had the lowest attachment of germs when compared to composite brackets and ceramic brackets. This is because metal brackets have a smoother surface texture than ceramic and composite brackets. Because ceramic and composite brackets are more porous, microorganisms can more easily attach to them.[18]

According to previous in vitro and in vivo studies,[10] coating orthodontic archwires prevents biofilm formation because the coating gives the archwire anti-adhesive qualities, which reduces the buildup of bacterial plaque. Yet, during fixed orthodontic therapy, the coated archwire's visible peeling that takes place under in vivo settings can cause bacteria to accumulate on the coated archwire's cracked surface. The patient's needs cannot be addressed by the aesthetic coated archwire if the white spot lesion develops as a result of an increase in cariogenic flora brought on by the buildup of microbial plaque on its surface. This will also have an unfavorable effect on the success of the treatments.[10]

Orthodontists can use the results of a study when deciding what kind of orthodontic appliance to use. Although lactobacillus is one of the many pathogenic organisms that accumulate and colonize in plaques, it does not play a significant part in the initial development of the lesion. Nonetheless, lactobacillus is crucial to the subsequent growth of the lesion. Prevention of lesions is something that orthodontists must pay attention to, because the resulting lesions are not aesthetically pleasing, unhealthy, and potentially irreversible.[15]

Study Limitations

This study has certain limitations such as it only used brackets made by one manufacturer. This may be expanded by including other bracket kinds, such as conventional and self-ligating brackets, or by contrasting ceramic and sapphire attractive brackets. Another limitation is the incubation period for the study was only 24 hours. In order to advance this research, it will be necessary to compare *Lactobacillus acidophilus* attachment on specific extended incubation times.

CONCLUSION

The ceramic bracket-coated NiTi archwire and the metal bracket-uncoated NiTi archwire showed significant differences in *Lactobacillus acidophilus* adhesion. *Lactobacillus acidophilus* had the highest adherence in the ceramic bracket and coated NiTi archwire group and the lowest adherence in the metal bracket and uncoated NiTi archwire group.

Acknowledgments

The authors are grateful to Hasanuddin University Hospital for allowing the researchers to carry out their research in their research laboratory.

Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

Author Disclosure

All authors declared no conflicts of interest.

Funding Source

This study has no funding support.

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