IDENTIFICATION OF THE NUMBER OF *STREPTOCOCCUS MUTANS* COLONIES ON SELF CURING ACRYLIC RESIN PLATES AND THERMOPLASTICS AS THE BASE MATERIAL OF REMOVABLE ORTHODONTIC RETAINER APPLIANCE

(Laboratory Experimental Research)

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Thesis

As one of the requirement to achieve bachelor's degree of

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at

BACHELOR OF DENTAL MEDICINE
DEPARTEMENT OF ORTHODONTICS
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Thesis,

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ABSTRACT

NIKE GITA ARMISWARI. **Identification of the number of Streptococcus mutans colonies on self-curing acrylic resin plates and thermoplastics as the base material of removable orthodontic retainer appliance (laboratory experimental research)** (supervised by Donald R. Nahusona, drg., M.Kes.Sp.Ort)

Background: Research shows that the use of orthodontic appliances can shift bacterial communities, increase biofilm accumulation, and potentially cause problems such as caries, periodontal disease, and enamel decalcification. S. mutans, an acid-producing bacteria that causes tooth decay, can be found in orthodontic appliance components. The properties of self-curing acrylic, which is widely used as the base of removable orthodontic retainers, tend to have high porosity, increasing the risk of bacterial adhesion and plaque colonization. The use of thermoplastic retainers has advantages in aesthetics but has disadvantages such as a tendency to loosen, discoloration, cracks, and favoring the growth of cariogenic bacteria due to the limited rinsing effect of saliva. The study aimed to determine the number of S. mutans colonies on self-curing acrylic resin plates and thermoplastic plates to consider the use of removable orthodontic retainer appliance bases. **Methods:** The type of research used was in the form of laboratory experimental research with a post-test-only-controlled group research design to calculate the number of colonies of S. mutans bacteria using a colony counter tool with CFU/ml units. **Results:** The average value of the number of S. mutans colonies growing on BHIA media, the result of immersion in self-curing acrylic resin plates was 467,20 $\times 10^{-1}$ CFU/ml with a standard deviation of 283,93$\times 10^{-1}$ CFU/ml Meanwhile, the average value of the number of S. mutans colonies growing on BHIA media, the result of immersion in thermoplastic plates was 105,40$\times 10^{-1}$ CFU/ml with a standard deviation of 67,96$\times 10^{-1}$ CFU/ml. **Conclusion:** There is adhesion of S. mutans colonies to self-curing and thermoplastic acrylic resin plates. The number of attachment of S. mutans colonies is greater on self-curing acrylic resin plates than thermoplastics. There was a significant difference in the number of Streptococcus mutans colonies between the self-curing and thermoplastic groups.

Key word : Streptococcus mutans, self curing acrylic resin, thermoplastic, removable orthodontic retainer

ABSTRAK

NIKE GITA ARMISWARI. ***Identifikasi jumlah koloni Streptococcus mutans pada lempeng resin akrilik self curing dan termoplastik sebagai bahan basis peranti retainer ortodonti lepasan (penelitian eksperimental laboratoris)*** *(dibimbing oleh Donald R. Nahusona, drg., M.kes., Sp.Ort.)*

Latar belakang: *Penelitian menunjukkan bahwa penggunaan peranti ortodonti dapat menggeser komunitas bakteri, meningkatkan akumulasi biofilm, dan berpotensi menyebabkan masalah seperti karies, penyakit periodontal, dan dekalsifikasi enamel. S. mutans, bakteri penghasil asam yang menyebabkan kerusakan gigi, dapat ditemukan pada komponen peranti ortodonti. Sifat akrilik self curing yang banyak digunakan sebagai basis peranti retainer ortodonti lepasan cenderung memiliki porositas tinggi, meningkatkan risiko adhesi bakteri dan kolonisasi plak. Penggunaan retainer termoplastik memiliki kelebihan dalam estetika namun memiliki kelemahan seperti kecenderungan longgar, perubahan warna, retakan, dan mendukung pertumbuhan bakteri kariogenik karena keterbatasan efek pembilasan dari air liur. Penelitian bertujuan untuk mengetahui jumlah koloni S.mutans pada lempeng resin akrilik self curing dan lempeng termoplastik untuk mempertimbangkan penggunaan basis peranti retainer ortodonti lepasan.* ***Metode:*** *Jenis penelitian yang digunakan berupa penelitian eksperimen laboratoris dengan desain penelitian post-test-only-controlled group untuk menghitung jumlah koloni bakteri S. mutans menggunakan alat colony counter dengan satuan CFU/ml.* ***Hasil:*** *Nilai rerata jumlah koloni S. mutans yang tumbuh pada media BHIA, hasil perendaman lempeng resin akrilik self curing sebesar* $467{,}20 \times 10^{-1}$ *CFU/ml dengan standar deviasi sebesar* $283{,}93 \times 10^{-1}$ *CFU/ml. Sedangkan nilai rerata jumlah koloni S. mutans yang tumbuh pada media BHIA, hasil perendaman lempeng termoplastik adalah* $105{,}40 \times 10^{-1}$ *CFU/ml dengan standar deviasi sebesar* $67{,}96 \times 10^{-1}$ *CFU/ml.* ***Kesimpulan:*** *Terdapat perlekatan koloni S. mutans pada lempeng resin akrilik self curing dan termoplastik. Jumlah perlekatan koloni S. mutans lebih banyak pada lempeng resin akrilik self curing daripada termoplastik. Terdapat perbedaan signifikan dalam jumlah koloni Streptococcus mutans antara kelompok self curing dan termoplastik.*

Kata kunci : *Streptococcus mutans, resin akrilik self curing, termoplastik, retainer ortodonti lepasan*

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CHAPTER I
INTRODUCTION

1.1. Background

In orthodontic treatment, retention appliance are indispensable to prevent teeth from returning to their original position. An orthodontic retainer is a passive appliance to maintain a corrected tooth in its new location (Kurniadi & Susilowati, 2018). Retainers are most often used after orthodontic treatment to keep the teeth in position while providing an opportunity for the surrounding tissues to re-form and to maintain the teeth in their ideal functional and aesthetic relationship and to prevent the tendency of the teeth to return to their original position (Vignesh & Sumathifelicita, 2015).

Changes in the oral environment such as placing orthodontic brackets or wearing orthodontic appliances can shift the bacterial community from healthy to one that can cause disease (Kitada et al., 2009). More than 700 types of bacteria coexist within the normal human oral cavity. These microorganisms are made up of the genus *Lactobacillus, Streptococcus, Eubacteria, Fusobacterium, Capnocytophaga, Staphylococcus, Eikenella, Porphyromona, Leptotrichia, Prevotella, Peptostreptococcus, Treponema,* dan *Actinomyces*. Microorganisms within the oral cavity can change as a result of changes in the oral cavity environment, especially in individuals wearing removable orthodontic appliances, regardless of type. S. mutans and L. acidophilus are the most common bacteria found in dental caries associated with the use of orthodontic appliances (Al-Lehaibi et al., 2021). Orthodontic treatment can lead to increased biofilm accumulation due to bacterial retention in orthodontic components. This increase in accumulation and poor oral hygiene can lead to increased caries, periodontal disease, and enamel decalcification (Velliyagounder et al., 2022)

S. mutans is an acid-producing bacterium that colonizes the surface of the teeth. These bacteria cause damage to the hard tissues of the teeth due to the fermentation of carbohydrates, such as sucrose and fructose. The biofilm layer is a slimy layer consisting of millions of bacterial cells, saliva polymers, and food debris. The layer of biofilm that has reached a certain thickness is called plaque, then the plaque will provide an excellent adhesion site for colonization and growth of bacteria (Evangelina et al., 2021). A systematic review found that the levels of this bacteria increased during orthodontic treatment, and three months after the removal of the appliance there was a significant decrease in the number of these pathogens (Velliyagounder et al., 2022).

There are several types of retention appliances, including: removable retention appliances made of acrylic with various modifications, flexible retention appliances, and transparent retention appliances (*clear retainer*). The removable orthodontic appliance has 3 main components, namely the active

component, the passive component and the base plate. The base plate is made of self curing or heat curing acrylic resin material and is used to hold active and retentive wire components in one piece (Goenharto & Rusdiana, 2015). The formation of acrylic resin occurs when monomers and polymers are mixed and the polymerization process occurs until PMMA is formed. PMMA composed of methyl metacrylate (MMA) monomer will connect and form a long polymer bond. PMMA polymerization causes porosity in its bulk structure depending on the degree of conversion of monomers to polymers (Juwita et al., 2018). Studies have shown that self-curing acrylic resin has more porosity than heat curing acrylic resin due to the low level of polymerization in self-curing acrylic which will ultimately lead to higher water absorption and an increased risk of bacterial adhesion and plaque colonization (Esmaeilzadeh et al., 2022).

In recent times, the use of thermoplastic retainers has increased due to their easy manufacture and good aesthetics (Akgün et al., 2019). Thermoplastic retainer appliance are made of a variety of different polymer materials whose manufacture involves heating the thermoplastic sheet followed by a vacuum procedure on top of the working model, which can permanently change the morphology of the material and its thermal properties (Albilali et al., 2023; Belayutham et al., 2023). Thermoplastic retainer appliances cover all palatal, lingual, labial, and buccal surfaces and gingivas. However, this appliance has disadvantages, namely loosening over time, discoloration, cracks, and causing limited rinsing and buffering effects of saliva on teeth and soft tissues, thus supporting the growth of cariogenic bacteria (Türköz et al., 2012). Thermoplastic retainers also have properties and surface roughness, surface energy, surface hydrophobicity that can affect bacterial adhesion (Al-Lehaibi et al., 2021).

Therefore, the researcher intends to determine the number of *S. mutans colonies on* self-curing acrylic resin plates and thermoplastic plates which can then be used as a consideration in selecting the base of removable orthodontic retainer appliances.

1.2. Problem Formulation

Based on the background that has been described, the problem can be formulated, namely: Are there *Streptococcus mutans colonies* on self-curing acrylic resin plates and thermoplastics as the base material for removable orthodontic retainer appliance?

1.3. Research Objectives

General Objectives

To determine the adhesion of *Streptococcus mutans colonies* to self-curing acrylic resin plates and thermoplastics as the base material of the removable orthodontic retainer appliance

Special Objectives

1. Counting the number of *Streptococcus mutans colonies* on self curing acrylic resin plates
2. Counting the number of *Streptococcus mutans colonies* on thermoplastic plates
3. Knowing the difference in the number of *Streptococcus mutans colonies* on self-curing *and thermoplastic* acrylic resin plates

1.4. Research Purpose

Scientific Purpose

1. Providing information on the number of attachment of *streptococcus mutans* colonies to *self-curing* acrylic resin plates and thermoplastics as a consideration in selecting the retainer base of the removable orthodontic appliance
2. Increasing the insight, knowledge, and experience of researchers, especially in the field of dentistry

Clinical Purpose

Useful for dentists in providing instructions and advice to patients in the selection and use of the base material of the removable orthodontic retainer appliance

Enviromental Purpose

Providing information to users of removable orthodontic retainer appliances on the importance of maintaining oral cavity hygiene and removable orthodontic retainer appliances

CHAPTER II
RESEARCH METHODS

2.1. Type of Research

The type of research used is laboratory experimental research with a *post-test-only-controlled group design*

2.2. Time and Location of Research

2.2.1. Location

1. Laboratory of Dental Material Faculty of Dentistry Hasanuddin University
2. Laboratory of Microbiology Faculty of Pharmacy Muslim University of Indonesia

2.2.2. Time

The research was conducted from January 2024 – March 2024

2.3. Research Variables

2.3.1. Independent Variable

The independent variables of this study were self-curing acrylic resin plates and thermoplastics

2.3.2. Dependent Variable

The dependent variable of this study is the attachment of *Streptococcus mutans* colonies

2.3.3. Controlled Variable

The control variables of this study were the type of plate used, the duration of contact, and the room temperature

2.4. Operational Definition

2.4.1. Self curing acrylic resin

Self curing *acrylic resin* is an acrylic resin material whose polymerization process is obtained from room temperature and mixed according to the manufacturer's instructions to form a plate with a size of 20x10x1 mm.

2.4.2. Thermoplastics

Thermoplastic is a sheet that is heated and compressed in vacuum equipment according to factory instructions and then cut into 20x10x10 mm wide using scissors

2.4.3. *Streptococcus mutans adhesion*

The attachment of *S. mutans* colonies can be determined by calculating the number of *S. mutans* colonies from the results *of S. mutans* shedding attached to *self-curing* acrylic resin plates and thermoplastics after being soaked in *tubes containing S. mutans* which are then planted on BHIA media. The number of colonies is expressed in units *of Colony Forming Units/milliliters (CFU/ml).*

2.5. Research Population and Sampel

2.5.1. Research sampel

The samples used in this study were *self-curing* acrylic resin plates and thermoplastics.

2.5.2. Research sample size

For laboratory experimental research with a post-test only control group design, the commonly used sample size formula is based on **the test of the difference between the two groups**, which is:

$$n = \frac{2(Z_{\frac{\alpha}{2}} + Z_{\beta})^2 . \sigma^2}{\Delta^2}$$

n = Sample size per group

$Z_{\frac{\alpha}{2}}$ = Z-score for confidence level (e.g., 1.96 for 95% confidence level)

Z_{β} = Z-Score value for Power (e.g., 0.84 for 80% Power)

σ = standard deviation of the combined population of both groups

Δ = expected difference between the mean colonies of Streptococcus mutans in both groups

$$n = \frac{2(1{,}96 + 0{,}84)^2 . 4{,}90^2}{5^2}$$

$$n = \frac{2(7{,}84)^2 . 24{,}01}{25}$$

$$n = \frac{15{,}68\ x\ 24{,}01}{25}$$

$$n = 15{,}05$$

Therefore, the number of samples per group is 15 after being rounded.

2.5.3. Sampel Criteria

1. **Inclusion criteria**
   - Self curing acrylic resin plates
   - Thermoplastic plates
   - Plate size 20x10x1 mm
2. **Exclusion criteria**
   - Porous acrylic resin plates
   - Thermoplastic plates that are crazing or deformed

2.6. Research Instruments

2.6.1. Tools

The tools used in this research are;

1. Wax knife
2. Glass plate
3. Cuvette
4. Rubber bowl and spatula
5. Vibrator
6. Scissors
7. Digital scale
8. Magnetic stirer
9. Autoclave
10. Ose
11. Forceps
12. Erlenmeyer flask
13. Rack and test tube
14. Incubator
15. Colony counter

2.6.2. Materials

The materials used in this materials are;

1. Base plate wax
2. Dental plaster
3. Water
4. Thermoplastic sheet
5. Handscoon
6. Mask
7. Cotton skewers
8. Self—curing acrylic resin
9. Syringe 2 or 3 cc
10. Tissue
11. 9,8 gram $NaHCO_3$
12. 10 gram Na_2HPO_4 $12H_2O$
13. 0,57 gram KCl
14. 0,47 gram NaCl
15. 0,12 gram $MgSO_4$ $7H_2O$
16. 5,3 gram $CaCl_2$
17. HCl 0,1 m
18. *Streptococcus mutans* stock
19. PZ solution (*Physiological Zouth*)
20. BHIB Medium (*Brainheart Infusion Broth*)
21. BHIA Medium (*Brainheart Infusion Agar*)
22. PBS (*Phosphate Buffered Saline*)

2.7. Research Procedure

2.7.1. Manufacturing of self curing and thermoplastic plates

The first preparation stage is the manufacture of *self-curing* acrylic resin and thermoplastic plates with a size of 20x10x1 mm of 15 pieces each.

Manufacturing procedure of self curing *acrylic resin plates* *(Manappallil, 2016)*

1) Cut 15 pieces of baseplate wax with size 20x10x1 mm using a wax knife on a glass plate
2) Malam merah yang sudah dibentuk ditanam dalam kuvet dengan dental plaster sambal divibrasi. Tunggu sampai dental plaster setting, kemudian keluarkan malam dengan hati-hati. The formed baseplate wax is inserted in a cuvette with dental plaster while vibrating. Wait until the dental plaster setting, then remove the wax carefully.
3) Next, sprinkle the acrylic powder little by little evenly. Then drip the liquid until the entire acrylic powder is absorbed by the liquid, then vibrate. Do it until the entire part is covered with acrylic resin material with a thickness of 1 mm.
4) The resin plate is separated from the cast by using a small saw or cast blade carefully so as not to be deformed.
5) A good acrylic resin plate is non-shaft and shiny.

Manufacturing procedure of thermoplastic plates (Albilali et al., 2023)

1) Take the thermoplastic sheet and then vacuum it according to the manufacturer's instructions using a vacuum pressed
2) Sheets of thermoplastic that have been pressed, cut into 20x10x1 mm widths using scissors.

2.7.2. Making Saliva Buffer (Suningsih et al., 2017)

1) Weight accurately 9,8 gram $NaHCO_3$, 10 gram Na_2HPO_4 $12H_2O$, 0,57 gram KCl, 0,47 gram NaCl, 0,12 gram $MgSO_4$ $7H_2O$.
2) It is then dissolved with 500 ml of aquades in a goblet glass (1000 ml capacity) (Solution 1). The dissolution is carried out at a temperature of 39°C and uses a magnetic stirrer to speed up the process.
3) Next, 5.3 grams of $CaCl_2$ were accurately weighed then put into a measuring cup and dissolved with 100 ml of aquades (Solution 2).
4) Then solution 2 is added to solution 1, stirring until homogeneous (Solution 3)
5) Next, aquades are added to the solution of 3 until the volume becomes 1000 ml, then 1 liter of saliva buffer solution is formed.
6) To neutralize the pH, 0.1 m HCl is added to the solution. The HCl solution was prepared by diluting 455.75 ml of concentrated HCl (normality 11.3) with 44.25 ml of aquades.

2.7.3. Identification and making of *Streptococcus mutans suspension* *(Zettira et al., 2017)*

The *S. mutans bacteria* that will be used in the research are taken from the stock *of S. mutans* at the Microbiology Laboratory, Faculty of Pharmacy, Muslim University of Indonesia. The manufacture of *S. mutans* was obtained by taking 2 ml of PZ solution plus 1 ose of *S. mutans* bacteria and then

putting it in a desicator for 24 hours, then the turbidity level was seen on the spectrophotometer in accordance with the Mc Farland standard no.1 (3×108 CFU/ml) with a wavelength of 560 nm.

The bacterial medium is made from 3.7 grams of BHI-B powder, 100 cc of aquades are added to Erlenmeyer, then heated in boiling water, chili sauce stirred with a spatula until homogeneous. It is then sterilized in an autoclave at 121°C for 15 minutes.

2.7.4. Treatment stages (Maharani et al., 2017; Zettira et al., 2017)

Samples are pre-sterilized in an autoclave at 121°C for 15 min. Then the sample was soaked in saliva buffer for 1 hour and then rinsed with PBS solution. Next, the sample was put in a test tube containing *a suspension of S. mutans* and then incubated for 24 hours. Samples were taken using sterile forceps and then inserted into a test tube containing 10 ml of sterile aquades and vortex for 1 minute to remove the *S. mutans* bacteria attached to the surface of the sample. Dilution is performed by transferring 1 ml from the first test tube to a sterile test tube containing 9 ml of aquaades then repeated until dilution $^{10\text{-}5}$. Next, S*. mutans* was bred by taking 0.1 ml of the vortex results of each sample and putting it into BHIA in a petri dish then flattened using a spreader and then incubated at 37°C for 2×24 hours.

2.7.5. Calculation of the number of *Streptococcus mutans* colonies

S*. mutans* bacteria growing on BHI solid media were calculated by dividing the surface of BHI solid media into 8 parts, then the number of S. mutans colonies growing on each part was calculated using a colony counter with units (CFU/ml).

2.7.6. Data analysis

The data obtained were analyzed by normality test with Shaphiro-wilk and homogeneity with Levene's test. If the data is normally distributed and homogeneous, the data is analyzed statistically using a parametric test with an independent t test to see the difference in the number of attachment of Streptococcus mutans colonies between self-curing and thermoplastic acrylic resin plates. However, if it is not distributed normally and homogeneously, the data analysis uses a non-parametric test with the Mann Whitney test.

2.8. Research Flow