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Attachment Differences of *Candida albicans* Colonies on Self Cured and Heat Cured Acrylic Resin Plates Used as Removable Orthodontic Appliances

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ABSTRACT

Acrylic resins are often used as bases for removable orthodontic appliances, both self-cured and heat-cured acrylic resins. One of the rough parts on the surface of the removable orthodontic appliances base is the part that faces the mucosa so that the sharp part easily triggers plaque adhesion and food scraps that will increase the colony of *Candida albicans*. This study aimed to determine whether there is a difference in the attachment of *Candida albicans* colonies between self-cured and heat-cured acrylic resin plates used as a removable orthodontic appliance's base material. This study was an experimental laboratory with a posttest only group design. The research samples were ten plates of each self-cured acrylic resin and heat-cured resin. Both types of ingredients were soaked in 10 ml of *Candida albicans* suspension. The results with an independent t-test showed a significant difference between the attachment of Candida albicans colonies on self-cured and heat-cured acrylic plates with a significance value of p = 0.012 (p <0.05).There was a difference in the attachment of *Candida albicans* colonies on self-cured and heat-cured acrylic resin plates used as removable orthodontic appliances base material.

Keywords: Acrylic resins, *Candida albicans*, heat-cured, orthodontic appliance, self-cured

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INTRODUCTION

Public interest in orthodontic treatment increases by the awareness of the importance of dental and oral health.[1] Orthodontic treatment aims to improve tooth structure, correct dento-craniofacial male relation, and malformation to restore the function of chewing, speaking, and facial appearance. This change is due to the use of devices that can move the teeth or modify the jaw growth.[2] Orthodontic appliances can be removable appliances, or fixed appliances.[3] Previous research has shown that using orthodontic appliances makes some intraoral changes, such as increased biofilm accumulation, increased accumulation of microbial colonization, enamel demineralization, changes in salivary buffer capacity, and even cause harmful effects on periodontal tissue.[4]

Candida albicans is a microorganism that is commonly found in the oral cavity with about 40% as a regular part. However, long-term use of orthodontic devices can create a favorable environment for enhancing *Candida albicans* colonies in the mouth.[4]

Removable orthodontic appliances consist of active components, passive components, and a base. The base is in the form of a curved plate following the palate or lingual surfaces, usually made of acrylic resin.[5,6] The acrylic resin consists of a powder (polymer) made from polymethyl methacrylate (PMMA) and a liquid (monomer) in the form of methyl methacrylate (MMA).[7] Inside the oral cavity, the surface of the acrylic resin as well as on the other parts of the oral cavity, is covered by saliva with high protein content. Saliva can get trapped in micropores on the base's surface, especially on the surface of the plate facing the mucosa where this part is rough / not polished, making it easier for plaque buildup and food scraps. This existence will facilitate the colony of microorganisms breeding in these places, including *Candida albicans*.[3,8]

Types of acrylic resin polymerization methods commonly used to make removable orthodontic appliances bases are self-cured and heat-cured acrylic resins.[6]Sundari et al. studied the differences in the average number of *Candida albicans* colonies between thermoplastic nylons with heat-cured acrylics due to the microporosity of acrylic resin easily triggers heaps of microorganisms on the surface compared to thermoplastic nylon.[9] The attachment of *Candida albicans* to the acrylic resin plate is influenced by the initial attachment of *Candida albicans* to the acrylic surface. Subsequently, it depends on the physical properties of the material surface, such as porosity, surface energy, hydrophobicity, and roughness, influenced by the acrylic resin polymerization method.[10] From the description above, the purpose of the study was to determine whether there are differences in the attachment of *Candida albicans* colonies on self-cured and heat-cured acrylic resin plates.

MATERIALS AND METHODS

This study was an experimental laboratory with posttest only control group design—the study conducted at the Microbiology Laboratory, Faculty of Medicine, Hasanuddin University. Samples used were acrylic resin plates, which were divided into two groups, namely self-cured acrylic resin plates group, and heat-cured acrylic resin plates group. Both groups consisted of 10 samples that met the sample criteria. The inclusion criteria for acrylic plates were not polished and measured 10x10x1 mm. Exclusion criteria were porous acrylic resin plates.

Manufacture of self-cured and heat-cured acrylic resin slabs

The procedure for making self-cured acrylic resin plates starts with cutting 10x10x1 mm dental waxes to 10 pieces and then implanting them in cuvettes with dental plaster. After setting, the wax was removed, and the acrylic powder is sown gradually, followed by incubating the liquid until the liquid absorbs all the acrylic powder. After polymerization, the acrylic resin plates were separated from the plaster.

As with the procedure for making self-cured acrylic resin plates, the heat-cured system also begins by preparing ten pieces of dental waxes, planted in a cuvette and then pressed. The boiling out process was carried out to get the mold chamber. The acrylic stirring process was carried out in ceramic glass; when it reached the dough stage, the acrylic was applied to the mold chamber. Cellophane plastic was placed between the top cuvette and the bottom cuvette and then pressed until there was no acrylic attached. After that, the acrylic was smeared with liquid, and the final press was done without cellophane plastic. The water was boiled, and then the cuvette was put for ± 1 hour 30 minutes in boiling water. Finally, the cuvette is lifted and allowed to cool down to room temperature, after which the cuvette was opened, and the acrylic resin plate was separated from the plaster.

Saliva buffer preparation

Saliva buffer was obtained by preparing the McDougall solution. The first weighed accurately: 9.8 grams of NaHCO3, 10 grams of Na2HPO4 12H2O, 0.57 grams of KCl, 0.47 grams of NaCl, 0.12 grams of MgSO4 7H2O. Furthermore, it was dissolved with 500 ml of distilled water in a glass cup (1000 ml capacity) (Solution 1). Dissolution was carried out at 39ºC and used a magnetic stirrer to speed up the process.

Then 5.3 grams of CaCl2 were accurately weighed and put into a measuring cup and dissolved with 100 ml of distilled water (Solution 2). After that, 1 ml of Solution 2 was added to Solution 1, stirring until it was homogeneous (Solution 3). Then distilled water was added to Solution 3 until the volume became 1000 ml, then formed 1 Liter McDougall solution. 0.1 m HCl was added to the solution to neutralize the pH. A 0.1m HCl solution was prepared by diluting 455.75 ml of concentrated HCl (normality 11.3) with 44.25 ml of distilled water. The results were collected in 1 test tube to make pellicle formation on the acrylic resin plate.

Candida albicans suspension preparation

Candida albicans suspension was made by taking one eye *Candida albicans* from seed in SDA media, then put into Sabouroud's broth 5 ml and incubated for 48 hours at 37°C. Furthermore, the suspension was taken from the previous as much as one inoculum needle and put into a test tube containing 5 ml of Sabouroud's broth, which will be contaminated with acrylic resin plates.

Treatment stage

All acrylic resin plates were sterilized in a 121° C autoclave for 18 minutes, then sterile saliva soaking for 1 hour, expected to form a pellicle on the resin plate. All the acrylic resin plates were rinsed with PBS solution two times then contaminated with *Candida albicans*. Each acrylic resin plate was inserted in a test tube containing a *Candida albicans* suspension incubated for 24 hours at 37°C.

After that, insert the acrylic resin plate into a tube containing Sabouroud's broth 10 ml and then vibrate for 30 seconds. Take 0.1 ml of *Candida albicans* suspension planted in SDA media then incubated for 48 hours at 37°C. The calculation was done using a colony counter in units of CFU / ml.

Data analysis

The data obtained were analyzed by the normality test with Shapiro-Wilk and homogeneity test with Levene's test. The data were statistically analyzed using the parametric analysis with an independent t-test.

RESULT

The attachment of *Candida albicans* colonies toward self-cured and heat-cured acrylic resin plates can be determined by counting the number of *Candida albicans* colonies growing on SDA media resulting from *Candida albicans* attachment to the acrylic resin plates after immersion in *Candida albicans* suspension.

Table 1 shows the mean value of *Candida albicans* colonies attachment on self-cured acrylic resin plates $1,939.3x10^{-1}$ CFU/ml with a standard deviation of $304.336x10^{-1}$ CFU/ml. At the same time, the mean in the heat-cured acrylic resin group was $1,536,9x10^{-1}$ CFU/ml with a standard deviation of $334.660x10^{-1}$ CFU/ml.

From the results of the study, a graph of the average histogram regarding *Candida albicans* colony attachment on a self-cured and heat-cured acrylic resin plate can be seen in Figure 1.

Figure 1 shows that the average attachment of *Candida albicans* colonies on self-cured acrylic resin plates is higher than the number of *Candida albicans* colonies on heat-cured acrylic resin plates.

Differential tests in the average attachment of *Candida albicans* colonies on self-cured and heat-cured acrylic resin plates were carried out with the Independent t-test (unpaired t-test). This test is a parametric data test that must meet the normality and homogeneity test. The results of the normality test are summarized in Table 1, shows that the group of self-cured and heat-cured acrylic resin plates have significance values of 0.053 and 0.196, respectively. It means that the data obtained by the attachment of *Candida albicans* colonies on self-cured acrylic resin and heat cured plates are normally distributed (p> 0, 05). The Levene test statistic value was 0.595, with a significance of 0.451, so it is concluded that data are homogeneously distributed (p> 0.05).

After fulfilling the normality and homogeneity tests, an independent t-test was performed. Independent t-test in this study examined differences in the attachment of *Candida albicans* colonies on self-cured and heat-cured acrylic resin plates, which can be seen in table 2. The results of the Independent t-test in table 2 showed a significant difference between the attachment of Candida albicans colonies in self-cured acrylic resin groups and heat-cured acrylic resin groups (p=0.012).

DISCUSSION

This research aimed to observe whether there were differences in the attachment of *Candida albicans* colonies on plates used as a removable orthodontic appliance's base material.

The results showed that the number of *Candida albicans* colonies in the group varied in each sample. This result may be due to differences in the quality of each sample, mainly because the acrylic resin plate's surface was not polished, which causes variations in roughness on the surface of the sample, so the samples obtained influences the calculation results of the *Candida albicans* colony. Also, differences in the number of colonies of *Candida albicans* may be caused by the suspension of *Candida albicans* obtained that was not the same between one plate to another.[11, 12]

The mean value of 10 pieces of self-cured and heat-cured acrylic resin plates contaminated with *Candida albicans* showed differences in the attachment of *Candida albicans* colonies in self-cured and heat-cured groups. This study shows the average number of *Candida albicans* attachments in the self-cured acrylic resin group was $1,939.3x10^{-1}$ CFU/ml, and heat-cured was $1,536.9x10^{-1}$ CFU/ml. Independent t-test analysis results showed that there was a significant difference ($p < 0.05$) where the attachment of *Candida albicans* colonies was greater in the group of self-cured acrylic resin compared to heat-cured ones.

Significant differences in the attachment of the *Candida albicans* colony are thought to be due to the different roughness on the surface of the self-cured and heat-cured acrylic resin plates. The surface of the self-cured acrylic resin plates is coarser than the heat-cured ones. This is in line with the result of Sundari et al., which states that there are differences in the average number of *Candida albicans* colonies between thermoplastic nylon and heat-cured acrylic, which is caused by micropores on acrylic resin plates which easily trigger piles of microorganisms on the surface compared to thermoplastic nylon.[9]

However, the results of this study are not in line with the study of Mubarak et al., about the comparison of *Candida sp.* colony volume with mouth rinses using denture, it was found that there was no significant difference in the number of *Candida sp.* colonies on the gargling volume of self-cured and heat-cured acrylic resin denture users. Nevertheless, it is said that clinically it still shows that there are differences in the number of colonies of *Candida sp.* in subjects using self-cured acrylic resin dentures containing more colonies of *Candida sp.* from the subject of heat-cured acrylic resin users.[13]

The study by Young B. et al. found differences in the attachment of *Candida albicans* to acrylic resins processed by three different methods. The results showed a significant difference ($p < 0.05$) between *Candida albicans* adhesion on denture base acrylic resin with a significantly reduced self-cured method compared with acrylic resin with conventional pressure packaging methods. However, the comparison of self-cured and packing pressure conventional to resin with injection molding methods did not show significant changes in *Candida albicans* adhesion to this acrylic resin ($p > 0.05$).[10] This is consistent with this study regarding the relationship between material roughness bases and the *Candida albicans* colony's adherence, both on removable orthodontic appliances and denture bases.

The rough surface of the orthodontic appliances is a favorable environment for the growth of microorganisms. The formation of biofilms depends on several factors, including bacterial species, their nature, and substrate, environmental factors as well as significant gene products.[4] Microorganisms attached to the surface of the base of the orthodontic appliance can proliferate to form plaques that will affect the state of the oral cavity and systemic health. Attachment of these microorganisms will cause bad breath, candidiasis, and various other complaints associated with orthodontic appliances.[13]

The base material itself also influences the growth of microorganisms on removable orthodontic appliances bases. In this research, the type of acrylic used is self-cured acrylic resin (chemically activated acrylic resin) and heat-cured (heat activated acrylic resin). Chemically activated and heat-activated ingredients have micropores, making it easier for food scraps and bacteria to get into them. Acrylic bases that come in direct contact with saliva, and absorb specific salivary molecules form a thin organic layer called the acquired pellicle. Pellicles contain proteins that bind microorganisms in the oral cavity so that organisms attach to the surface of the device base and multiply and colonize with other organisms to form orthodontic device plaques. Orthodontic appliances plaque is a cause of problems associated with periodontal tissue, discomfort, stomatitis, bad breath, and inflammation of the mucosal tissue beneath the device.[13] This is related to research conducted by Saleem AI. Regarding the use of removable top orthodontic appliances against *Candida* cultures in the oral mucosa. In his research, it was explained that there was an increase in the *Candida* culture in the mucosal area put on the device and around the area put on the dental appliances.[14]

CONCLUSION

There is a significant difference in the attachment of *Candida albicans* colonies on self-cured and heat-cured acrylic resin plates as a basis for removable orthodontic appliances. The number of *Candida albicans* colonies is higher in the self-cured acrylic resin plates than in heat-cured ones.

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Table 1. Average Data and Deviation Standards for Attachment of the *Candida albicans* Colony

Group	n	Mean ($x10^{-1}$ CFU/ml)	SD ($x10^{-1}$ CFU/ml)	p-value
Self-cured	10	1939.3	304.336	0.053*
Heat cured	10	1536.9	334.660	0.196*

*Shapiro Wilk: Level of significance p> 0.05

Table 2. Independent t-test results of the self-cured and heat-cured acrylic base.

	Levene's Statistic		p-value
	F	Sig.	
Equal variances assumed	0.595	0.451	0.012*
Equal variances not assumed			0.012

*the significance level of Independent t-test p<0.05.

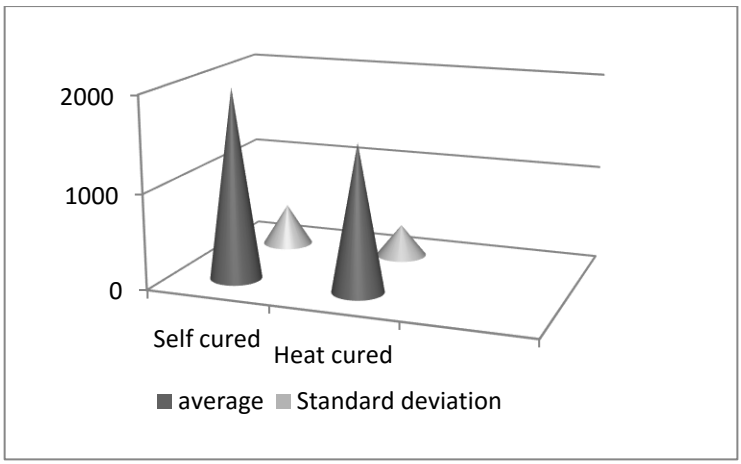

Figure 1. Average and Standard Deviation of *Candida Albicans* Attachment