Candida Albicans Biofilm Profiles on Various Denture Base Materials

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Abstract

The high prevalence of denture stomatitis caused by the use of dentures may influence the stability of oral candida. Assess the effects of two types of denture base material roughness on the attachment of *Candida albicans*. Performed roughness tests with a roughness tester and immersed dyed specimens into Eppendorf tubes containing a modified suspension of *C. albicans* incubated for 24 and 72 hours. The data were analyzed with a one-way analysis of variance (ANOVA) post-hoc test and bivariate correlation (Pearson). The amount of *C. albicans* colonization on the surface of the denture base decreased when polished compared to not polished. There were differences in the number of *C. albicans* colonies followed by a long incubation time. The number of colony forming units of *C. albicans* was influenced by a smoother surface of the denture base.

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Introduction

Denture stomatitis is a type of inflammation process in the oral mucosa. Various factors contribute to denture stomatitis, such as oral candida and other oral microorganism colonizations. Denture stomatitis occurs due to denture use with oral candida and other oral bacteria colonies on the base of the denture. This condition can also be caused by local and systemic factors, for example, the potential of hydrogen (pH) in the saliva and its degree of acidity, high carbohydrate consumption, long-term use of antimicrobial drugs, and diseases like diabetes mellitus and hypertension.[1] Previous study reported that denture stomatitis is caused by several factors. The primary causing factors are trauma from the denture and *Candida albicans* infection.[2]

Oral candida is a normal flora in the oral cavity, but other local and systemic factors may cause an overgrowth of the fungi. When the normal floras of oral mucosa are imbalanced or the host's immunity is reduced, then oral candida can turn into pathogens.[3]

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The most prevalent oral candida species found infecting humans is *C. albicans*. Among oral candida, *C. albicans* generally has a role in 50% of the cases of denture stomatitis.[4]

C. albicans has a high adhesive capacity in oral and vaginal epithelial cells. It has been reported that initially, *C. albicans* colonizes and invades the soft tissues of the oral cavity by adhesion on cells and the mucosa surface.[1]

Other factors that act in denture stomatitis formation other than systemic ones are local factors. One important local factor is the denture base, more specifically its material properties, whether physical or mechanical. The ideal material properties for a denture base are low water permeability, high mechanical strength, excellent color stability (so that when contaminated with liquid entering the oral cavity its color does not change), and resistance to liquid acids.[5]

Another local factor is the surface roughness of the denture base, especially the intaglio side, which has contact with the oral mucosa. The denture base surface facing the oral mucosa is usually rough since polishing is not done on that side.[2] Surface roughness in the denture base material is defined as an irregularity of the surface texture.[4]

This roughness aids in biofilm and food build-up, which helps increase *C. albicans* colonization and then causes denture stomatitis.[6]

Materials and methods

Specimens consisting of a metal alloy (cobalt-chromium), heat cured acrylic resin, and valplast were made in a T-shaped mold with 30 mm length, 4 mm width, and 4 mm thickness. The casting and polishing of the metal, acrylic resin, and valplast denture base specimens were done by using factory procedures. The surface roughness testing on each denture base material specimen was done using a surface roughness tester (Surftest SJ-301, Mitutoyo, Japan). The roughness value was stated by micrometer unit in roughness average (Ra). Ra is an average roughness value determined from the mean (midline) of the high and low points on a surface.[7] Statistically, this parameter is the most stable and redoable, and it works well when used with various kinds of surfaces.

The metal, acrylic resin, and valplast specimens were sterilized with alcohol (70%), aquadest, and chlorhexidine gluconate 0.2%. The specimens were first immersed in alcohol (70%) for 10 minutes, then in aquadest for another 10 minutes, and last in chlorhexidine gluconate 0.2% for 5 minutes. Afterwards, the specimens were rinsed with aquadest before being kept in glass bottles filled with aquadest until further use was needed. Eppendorf tubes (1.5 ml) were prepared for each specimen's immersion container. The tubes were modified by cutting a 4 x 4 mm square on each of the lids using blade number 12. Besides cutting the lid, the necks of the Eppendorf tubes were also cut to help the operator in taking out the T-shaped specimens from inside the tubes.

The *C. albicans* used in this research was retrieved from the stock of the Oral Biology Laboratory in the Faculty of Dentistry of Universitas Indonesia. The pure species of the *C. albicans* was tested by gram staining to prove its purity. The gram staining was done using methylene blue. After the purity of the *C. albicans* was confirmed, 1 ounce of the pure species was smeared on Sabouraud dextrose agar and then incubated for 24 hours in a temperature of 37 ^{o}C. After 24 hours of incubation, the colonies that had formed on the agar were tested using gram staining with methylene blue and observed with a microscope to prove the purity again. Afterwards, 1 ounce of the colony was placed on Sabouraud dextrose broth prepared in a 10 mL test tube, incubated for another 24 hours in 37^{o}C temperature, and later kept in the refrigerator with 4^{o}C temperature. The Sabouraud dextrose agar (SDA) and Sabouraud dextrose broth (SDB) were made according to factory guidelines. The data analysis was done using a bivariate correlation test (Pearson) to compare the *C. albicans*' colony forming unit (CFU) values in each specimen with different immersion times (24 and 72 hours). The bivariate test resulted in a correlation values that was significant (0.01).

Results

In the surface roughness tests, each material was found to have different surface roughness values. The test was done on two different groups of materials, polished and unpolished, for each material type of metal, acrylic resin, and valplast. A decrease in the average roughness value or Ra was observed in two specimen groups. Compared to the unpolished group, the roughness average value was lower in the polished group for each material type. Differences in the Ra of the metal, acrylic resin, and valplast surfaces in the polished and unpolished groups can be seen by a decrease in the Ra values. The difference in the Ra value of metal was as high as 71.42% or a decrease from 0.7 µm in the unpolished group to 0.2 µm in the polished group. Among the acrylic resin groups, the Ra value diverges almost 70% from 1.0 µm to 0.3 µm. The difference in the Ra value of the valplast groups was as high as 57.14% from 0.7 µm in the unpolished group to 0.3 µm in the polished group.

Graph 1 shows a comparison of the CFU values in the metal, acrylic resin, and valplast groups with different immersion times (24 and 72 hours). In the unpolished and polished metal groups with 24 hours of immersion time, a difference of the CFU values from 1.6 x 10^3 CFU/ml to 0.5 x 10^3 CFU/ml was found. In the acrylic resin groups, a value of 1 x 10^3 CFU/mL was seen in the unpolished surface group and 0.7 x 10^3 CFU/ml in the polished surface group. Among the Val past groups, a significant difference were found, from 180 x 10^3 CFU/ml for the unpolished surface group to 5 x 10^3 CFU/ml for the polished surface group. In the unpolished and polished metal groups with 72 hours of immersion time, a difference of CFU values from 48 x 10^3 CFU/ml to 0.6 x 10^3 CFU/ml was found. In the acrylic resin groups, there was an increase

of the values from 1.9 x 10^3 CFU/ml for the unpolished surface group and 47 x 10^3 CFU/ml for the polished surface group. Among the valpast groups, a significant difference was also found, from 98 x 10^3 CFU/ml for the unpolished surface group to 0.3 x 10^3 CFU/ml for the polished surface group. A comparison of the effects of immersion time on the unpolished surface of each material type can be viewed in Figure 1.

Figure 1 shows a decrease of *C. albicans* growth in the valplast material from 180 x 10^3 CFU/ml after 24 hours immersion time to 98 x 10^3 CFU/ml after 72 hours immersion time. In other comparisons, increases of *C. albicans* colonies were found on the metal and acrylic resin materials. On the metal material, the increase was from 1.6 x 10^3 CFU/ml after 24 hours immersion time to 48 x 10^3 CFU/ml after 72 hours immersion time. Meanwhile, the acrylic resin material showed an increase of CFU value from 1 x 10^3 CFU/ml to 1.9 x 10^3 CFU/ml.

In Figure 2, an increase of *C. albicans* CFU values on the metal denture base occurred from 0.5 x 10^3 CFU/ml to 0.6 x 10^3 CFU/ml and on the acrylic resin denture base from 0.7 x 10^3 CFU/ml to 47 x 10^3 CFU/ml among the different immersion times. Meanwhile, the *C. albicans* CFU value on the valplast denture base material with different immersion times showed a decrease from 5 x 10^3 CFU/ml to 0.3 x 10^3 CFU/ml. The data in Graphs 1 and 2 can be rearranged to see a correlation between the average surface roughness or Ra of each material with the CFU value of *C. albicans* on each denture base material type (metal, acrylic resin, and valpast) according to the incubation or immersion time (24 and 72 hours).

According to the bivariate correlation (Pearson) analysis of each material, a strong positive relationship was found between the polished metal's *C. albicans* CFU value in different incubation times (24 and 72 hours) with the correlation value of 1.000. For the acrylic resin material, a strong positive relationship was found between the polished material's *C. albicans* CFU value in the 24 hours incubation time with a correlation value of 1.000. However, in the 72 hours incubation time, the relationship was strongly negative with an correlation value of -1.000. As for the bivariate correlation (Pearson) analysis of the valplast material, a strong positive relationship between the polished material's *C. albicans* CFU value in both incubation times was found with a correlation of 1.000. The bivariate correlation (Pearson) analysis can be seen in the graphs in the discussion section.

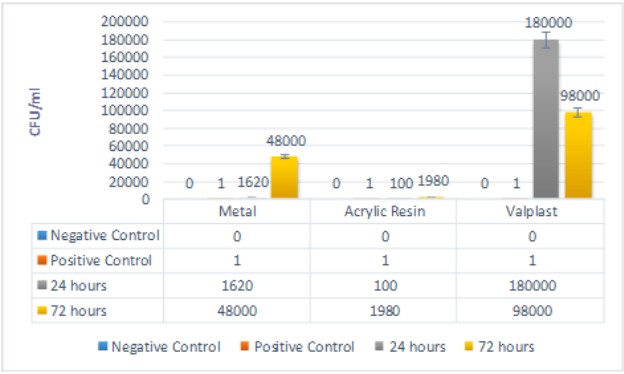

Figure 1. The CFU values of *C. albicans* colonies on unpolished denture base materials with 24 and 72 hours of immersion time.

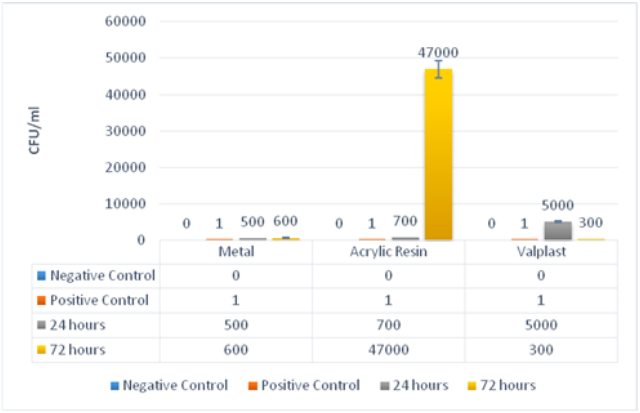

Figure 2. The CFU values of *C. albicans* colonies on polished denture base materials after 24 and 72 hours of immersion time.

Material	Ra	CFU value (24 hours)	CFU value (72 hours)
Metal (Unpolished)	0.7	1.6 x 10^3 CFU/ml	48 x 10^3 CFU/ml
Metal (Polished)	0.2	0.5 x 10^3 CFU/ml	0.6 x 10^3 CFU/ml
Acrylic resin (Unpolished)	1.0	1 x 10^3 CFU/ml	1.9 x 10^3 CFU/ml
Acrylic resin (Polished)	0.3	0.7 x 10^3 CFU/ml	47 x 10^3 CFU/ml
Valplast (Unpolished)	0.7	180 x 10^3 CFU/ml	98 x 10^3 CFU/ml
Valplast (Polished)	0.3	5 x 10^3 CFU/ml	0.3 x 10^3 CFU/ml

Table 1. The CFU value of *C. albicans* on each polished and unpolished material with different incubation times (24 and 72 hours).

Figure 3 shows an increase in the CFU values during the incubation period of 24 to 72 hours. This result is supported by biofilm theory stating that after 24 hours, *C. albicans* is still in the development phase toward maturation,

therefore the growth rate will still increase after 24 hours.[8]

Figure 4 shows an increase in the CFU values of *C. albicans* from 24 to 72 hours of incubation. The reason behind this is similar to the *C. albicans* biofilm theory stated above.[8] Besides biofilm theory, this might also be caused by the porous nature of acrylic resin, therefore *C. albicans* can easily adhere to the denture base.[9]

In the *C. albicans* cycle on polished acrylic resin, the CFU value is higher compared to its unpolished counterpart. This can be caused by the smoother surface of the denture base getting in the way of *C. albicans*' retention ability.[10] Thus after 24 hours, *C. albicans* still has the ability to develop and adhere onto denture base material up to the 72 hour incubation period.[8] Meanwhile, the CFU value on unpolished acrylic resin is lower than its value on polished acrylic resin. This may be because the rougher and more porous surface of the unpolished acrylic resin helps in increasing the growth rate of *C. albicans*.[10] Hence, the ability of *C. albicans* to grow after 24 hours on the polished acrylic resin is reduced.[8]

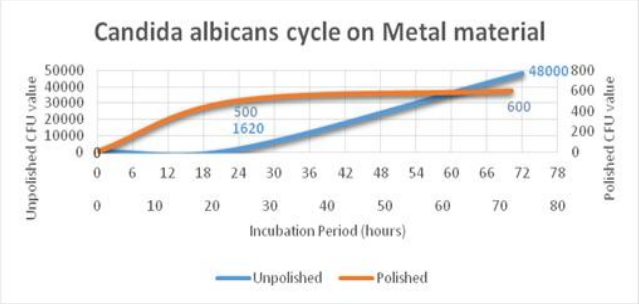

Figure 3. The *C. albicans* cycle on metal material.

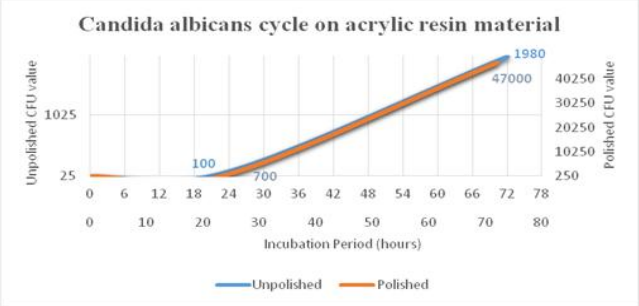

Figure 4. The *C. albicans* cycle on acrylic resin material.

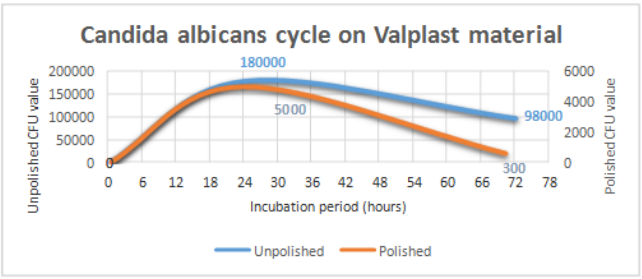

Figure 5. The *C. albicans* cycle on valplast material.

The *C. albicans* cycle on valplast denture base material illustrated in Graph 5 shows a decrease of CFU values from hour 24 to 72. This condition can be linked to valplast's less porous trait.[11] Therefore, *C. albicans* has difficulty sticking to the valplast denture base material.[8] From the bivariate correlation (Pearson) analysis of the metal, acrylic resin, and valplast denture base materials, there were strong positive relationships between metal polishing and the CFU value of *C. albicans* after 24 and 72 hours of incubation time with a correlation value of 1.000 each. On the acrylic resin, a strong positive relationship between polishing and the CFU value of *C. albicans* after 24 hours of incubation time was also found with a correlation value (R^2) of 1.000; however, a strong negative relationship was found after 72 hours of incubation time with a correlation value of -1.000. As for valplast, a strong positive relationship of polishing and the CFU value of *C. albicans* after 24 and 72 hours of incubation time was found with a correlation value of 1.000 each.

Discussion

This research was done to discover the effects of polishing three denture base materials (metal, acrylic resin, and valplast) on *C. albicans* CFU values. Both specimen groups of unpolished and polished materials were incubated for the same periods of 24 and 72 hours. This research was based on the supposition that unpolished denture bases have a huge influence on *C. albicans* growth compared to their polished counterparts.[7] The specimens used were different denture based materials specifically designed to form a T shape with 30 x 4 x 4 mm dimensions. The T shape of the specimens was made in this way to conform to the size of an Eppendorf tube.

The horizontal position of the T shape was placed outside the Eppendorf tube to maintain the stabilization of the specimens submerged in the Eppendorf tube. Prior to the experiment, the immersed part was standardized with specifications as follows: 7 mm immersed length and 4 mm immersed width and thickness. Therefore the surface areas of the immersed specimens were standardized and could be calculated with the formula $2\{(pl)+(pt)+(lt)\} = 2\{(7x4)+(7x4)+(4x4)\}$ $mm^2 = 144$ $mm.^2$

The Eppendorf tubes were 30.4 mm tall with a volume of 1.5 ml. The tubes were modified by making a 4 x 4 mm square hole on each lid using blade number 12. Further cuts were made on the neck of each Eppendorf tube so the specimens were easier to remove from the tubes. After the immersion of the denture base materials, 1 ml of SDB was poured into each Eppendorf tube. The measurement of 1 ml SDB poured into each tube was done according to how much the liquid rose when each specimen was put in the modified tube without the liquid pouring out. Denture stomatitis is found in 65% of denture users.[7] This research used *C. albicans* as a biofilm sample to be tested on metal, acrylic resin, and valplast denture base materials. The fungi species was chosen due to its main role in the development of denture stomatitis.[6] In denture use, candidiasis are exacerbated by accumulated *C. albicans* adhesion, therefore causing inflammation. Several studies have shown a strong correlation of denture base surface roughness with *C. albicans* adhesion.[6]

The *C. albicans* used in this experiment was incubated for 24 and 72 hours. In general, its growth occurs in three different development stages. In the initial phase, in the first 1-11 hours after microorganism introduction into an environment, *C. albicans* cells adhere to surfaces in 2 hours. Next, microcolonies appear after 3-4 hours. During the next 12-30 hours, *C. albicans* growth continues. After 38-72 hours, the colonies enter the last stage or the maturation phase. After 72 hours, the *C. albicans* is in its most optimum condition. This decided the incubation times for this experiment. The mediums for the *C. albicans* culture used in this research were SDA and SDB. These mediums are standard mediums containing the sugar and pepton used by fungi for its growth.[9,10]

The denture base materials used in this experiment were metal, acrylic resin, and valplast. The metal material consisted of a combination of cobalt and chromium due to their availability and cheaper costs than other metal materials. Acrylic resin was used since there are still many users of acrylic resin dentures. Valplast was chosen because of its non-rigidity, durability against abrasion, protection toward alveolar, and its good esthetics. Moreover, valplast denture base material has the advantage of being less porous.[10,11]

The results of this research were achieved by calculating each material's surface roughness using a roughness tester. With the roughness tester, an average value or Ra score was determined by making a mean line (midline) from the high (peak) and the low points of a surface.[10] Statistically, this parameter is the most stable and redoable. This parameter can also be used on any kind of surface. From this testing, the polished metal, acrylic resin, and valplast materials had lower surface roughness values than the unpolished denture base materials. The *C. albicans* suspension used in this experiment was tested for its purity and incubated on sterile SDA. The polished and unpolished metal, acrylic resin, and valplast materials were immersed in Eppendorf tubes filled with SDB until 7 mm of each specimen was fully submerged. From the culture of the *C. albicans* suspension on SDA, one colony was put in each Eppendorf tube containing SDB and incubated for 24 and 72 hours.

Conclusions

A decrease in the CFU value of *C. albicans* is affected by a decrease in the surface roughness value after the polishing of metal, acrylic resin, and valplast denture materials. Further field studies on the relationships between surface roughness, the permeability of the three different denture base materials, and microorganism adherence are needed. Additional studies on the influence of the surface roughness of denture base materials and the CFU values of *C. albicans* colonies are also needed. Clinical experiments on the correct cleaning methods or materials for denture bases may also be useful.

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