

## The Amount of *Streptococcus mutans* Biofilm on Metal, Acrylic Resin, and Valplast Denture Bases

Sheila Silvia<sup>1</sup>, Ariadna Adisattya Djais<sup>1\*</sup>, Sri Angky Soekanto<sup>1</sup>

1. Department of Oral Biology, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.

### Abstract

The prevalence of denture stomatitis is high in denture wearers. The objective of this study was to measure the effect of the surface roughness of denture base materials on the amount of *Streptococcus mutans* present.

The surface roughness was measured using a surface roughness tester. The specimens were dipped into an Eppendorf tube containing *Streptococcus mutans* and incubated for 12 and 24 hours. A bivariate Pearson correlation test was performed on the data.

There is a strong positive correlation between the surface roughness of the denture base material and the amount of *Streptococcus mutans*.

A decrease in the value of the surface roughness after polishing a metal, acrylic resin, or valplast denture base is followed by a decrease in the amount of *Streptococcus mutans*.

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### Introduction

Denture stomatitis, also known as denture sore mouth, is an inflammation of the oral cavity mucosa, primarily the palatal and gingival mucosa that are in direct contact with denture base. The condition is characterized by the presence of atrophy (erythemic lesions) and hyperplastic lesions.<sup>1,2</sup> Pachava et al. reported that the prevalence of denture stomatitis has been measured by different researchers as 23%, 39%, and 62%.<sup>1</sup> Broadly speaking, there are two causal factors of denture stomatitis: systemic factors and local factors.<sup>1,2</sup> The systemic factors are a high carbohydrate intake, long-term antibiotic therapy, hormonal therapy, and systemic conditions such as diabetes mellitus, hypertension, nutritional deficiencies (of iron, folic acid, or vitamin B12), hypothyroidism, immunocompromising conditions (e.g., HIV infection), malignancies (e.g., acute leukemia, agranulocytosis), and the use of iatrogenic

immunosuppressive medicines such as corticosteroids.<sup>1,2</sup> The local factors are the properties of the denture base and the microorganisms attached to the surface of the denture base.<sup>1,2</sup>

Denture base properties such as porosity, surface charge, amount of surface free radicals, hydrophobicity, and surface roughness have been reported to influence the attachment of microorganisms to denture bases.<sup>1</sup> Denture base surfaces that are in contact with the oral cavity mucosa are not polished; thus, more microorganisms are attached to an intaglio than a polished surface. Pachava et al. explained that rough or uneven denture bases facilitate the adhesion of fungi, and this condition complicates bacterial elimination during mechanical or chemical cleaning of the dentures.<sup>1</sup> The second local factor causing denture stomatitis is the microorganisms attached to the denture base. The first bacteria to attach to the denture base and form a colony are the Gram-positive bacteria *Streptococcus mutans*, which belong to the *Streptococcus* sp. group.<sup>2</sup> *S. mutans* synthesizes sticky extracellular polysaccharide substrates such as dextran or levan from sucrose.<sup>3,4</sup> These substrates are pathways for bacteria and other fungi to attach to denture bases. Bacteria and fungi will then colonize and proliferate into

#### \*Corresponding author:

Ariadna Adisattya Djais  
Department of Oral Biology  
Faculty of Dentistry, Universitas Indonesia,  
Jl. Salemba Raya No. 4 Jakarta Pusat  
E-mail: ariedjais26@gmail.com

biofilms on denture bases, leading to denture stomatitis.<sup>5</sup>

Denture bases can be made of various materials such as metal, acrylic resin, and valplast. The most commonly used metal for denture bases is a combination of cobalt and chromium (Co-Cr).<sup>6</sup> Another material that is commonly used is acrylic resin; Craig et al.<sup>7</sup> reported that 98% of denture bases are made of acrylic resin, also known as poly (methyl methacrylate), or PMMA.<sup>7</sup> Biofilms can easily attach to acrylic resin denture bases because of the porosity of the surface.<sup>6</sup> Besides metal and acrylic resin, the other material that can be used for denture bases is valplast. Valplast is a polyamide resin made of nylon. The surface roughness of valplast is higher than that of acrylic resin and facilitates the attachment of biofilms to and the discoloration of denture bases.<sup>8</sup>

A high surface roughness of the denture base and the presence of *S. mutans* are predisposing factors for denture stomatitis.<sup>1,2</sup> Al Kheraif<sup>9</sup> reported that a clinically acceptable value of denture base surface roughness is 0.2  $\mu\text{m}$ . If the surface roughness is greater than that, a proportional increase in biofilm accumulation will occur.<sup>9</sup> Further research on the amount of *S. mutans* in biofilms on denture bases made of metal, acrylic resin, and valplast is necessary because of the reasons previously stated.<sup>9</sup> The differences in the properties of denture bases made of metal, acrylic resin, and valplast could affect the ability of bacteria and fungi to colonize and proliferate to form biofilms that will lead to denture stomatitis.<sup>1</sup> In this *in vitro* study, the author hypothesized that there would be differences in the amount of *S. mutans* colonies on three denture base materials: metal, acrylic resin, and valplast.

### Materials and methods

A T-shaped metal specimen (Co-Cr alloy), a heat-cured acrylic resin specimen, and a valplast specimen were created with two parts: the holder and the part to be inserted into a block-shaped Eppendorf tube. The length of the holder was 30 mm. The standard size of the part of each specimen immersed in the TYS20B liquid media in the Eppendorf tube was 7 mm length by 4 mm width by 4 mm thickness. The surface area of the immersed parts was 144  $\text{mm}^2$ .<sup>2</sup> The total number of specimens used in the study was 6,

and the specimens were used in 4 groups. Groups 1 and 2 had 6 specimens each, while Groups 3 and 4 had 3 specimens each.

The surface roughness tests for the metal, acrylic resin, and valplast specimens were performed using a SurfTest SJ-301 surface roughness tester (Mitutoyo, Japan). The tester had a cutting length ( $\lambda_c$ ) of 0.25 mm and  $N = 5$ , so the evaluation length used ( $l$ ) was  $0.25 \text{ mm} \times 5 = 1.25 \text{ mm}$ . This tool has the ability to measure small variations on the surface by moving a diamond stylus transversally on the specimen surface under constant pressure.<sup>9,10</sup> The vertical displacement of the stylus measures the surface variability. The height position of the diamond stylus is converted into a digital signal that can be stored and processed. The surface roughness value is measured using an arithmetical roughness average (Ra) parameter in units of  $\mu\text{m}$ .<sup>11</sup>

The metal, acrylic resin, and valplast specimens were sterilized by immersing them in 70% alcohol for 10 min., aquadest for 10 min., and 0.2% chlorhexidine gluconate for 5 min. Afterwards, the specimens were rinsed with aquadest and stored inside a glass bottle containing aquadest until use.<sup>12,13</sup> A 1.5-ml Eppendorf tube was prepared as an immersion container for all the specimens. A modification was made to the tube to give it the ability to support a T-shaped specimen securely. The middle part of the tube lid was perforated with a scalpel to make a rectangular hole 4 mm long. The connector between the lid and the body of the tube was cut off with a scalpel.

The *S. mutans* used in this research was obtained from the inventory of the Oral Biology Laboratory of Dentistry of Universitas Indonesia. Pure isolates of *S. mutans* were examined for purity using Gram coloring, which was performed using crystal violet liquid, lugol, alcohol, and safranin. After being declared pure, a loop of *S. mutans* was taken and used to make a stock strain stroke in TYS20B agar media. The media was inserted into an anaerobe containment system in an incubator at 37°C for 48 hours.<sup>14</sup> After the incubation, the purity of the stock was examined again with the same Gram coloring technique as before and observed under a microscope. After being declared pure, one loop from the stock solution was placed in a modified Eppendorf tube, which was then filled with 1 mL of TYS20B liquid media.

The materials used to prepare the media were 200 g sucrose, 10 g yeast extract, 40 g trypticase soy agar, 5 g bacto agar, and 4mg/0.004g (200 UI) bacitracin. The materials were weighed and put into Erlenmeyer flask to which 1000 mL of sterilized aquadest was added. Homogenization was conducted, and the flask was then heated on a hot plate until the liquid boiled. The flask was then sterilized in an autoclave at 121°C for 15 minutes. The material was cooled and placed in a Petri dish that was made aseptic using a Bunsen burner [15]. The materials used to prepare the media were 200 g sucrose, 10 g yeast extract, 40 g trypticase soy agar, 5 g bacto agar, and 4mg/0.004g (200 UI) bacitracin. The materials were weighed and put into Erlenmeyer flask to which 1000 mL of sterilized aquadest was added. Homogenization was conducted, and the flask was then heated on a hot plate until the liquid boiled. The flask was then sterilized in an autoclave at 121°C for 15 minutes.<sup>15</sup>

Group 1 is the negative control group consisted of polished and unpolished metal, acrylic resin, and valplast specimens that were immersed in a modified Eppendorf tube containing 1 mL of TYS20B liquid media without a *S. mutans* suspension and incubated for 12 hours and 24 hours. Group 2 is the positive control group consisted of polished and unpolished metal, acrylic resin, and valplast specimens that were immersed in a modified Eppendorf tube containing 1 mL of TYS20B liquid media that had been inoculated with 1 loop of *S. mutans*. Group 3 is the treatment group consisted of unpolished metal, acrylic resin, and valplast specimens that were immersed in a modified Eppendorf tube containing a *S. mutans* suspension and incubated for 12 hours and 24 hours. Group 4 is the treatment group consisted of polished metal, acrylic resin, and valplast specimens that were immersed in a modified Eppendorf tube containing *S. mutans* and incubated for 12 hours and 24 hours.

## Results

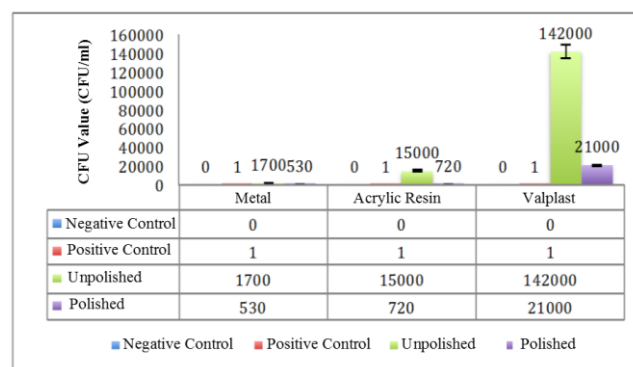
The surface roughness test results can be seen in Table 1. Both tables show the surface roughness value in terms of the Ra parameter of the polished and unpolished specimens.

Material	Specimen Surface	Unpolished Specimens Surface	Polished Specimens Surface
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		Roughness (µm)	Roughness (µm)
Metal	1	0.1 ± 0.01	0.3 ± 0.02
	2	0.1 ± 0.01	0.2 ± 0.02
	3	0.8 ± 0.06	0.2 ± 0.01
	4	1.2 ± 0.1	0.2 ± 0.01
	5	0.8 ± 0.03	0.3 ± 0.01
	Mean Total ±SD		0.6 ± 0.48
Acrylic Resin	1	0.2 ± 0.06	0.1 ± 0.01
	2	0.3 ± 0.08	0.1 ± 0.0
	3	1.0 ± 0.07	0.1 ± 0.02
	4	0.9 ± 0.18	0.1 ± 0.04
	5	1.5 ± 0.22	0.6 ± 0.1
	Mean Total ±SD		0.8 ± 0.54
Valplast	1	0.8 ± 0.19	0.7 ± 0.05
	2	0.8 ± 0.02	0.7 ± 0.09
	3	1.2 ± 0.08	0.6 ± 0.15
	4	1.0 ± 0.11	0.5 ± 0.06
	5	0.9 ± 0.05	0.7 ± 0.04
	Mean Total ±SD		0.9 ± 0.17

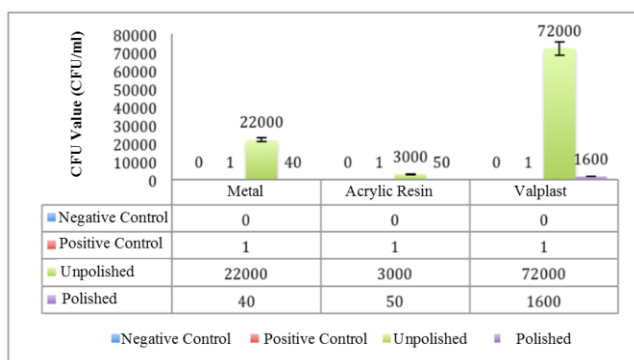
**Table 1.** The surface roughness of unpolished specimens as measured by a surface roughness tester (Surftest SJ-301, Mitutoyo, Japan).

A decrease in the surface roughness was observed for every material after being polished, as shown in Tables 1. The comparison of the unpolished and polished metal specimens showed a reduction in the surface roughness of up to 66.7%, from 0.6 µm to 0.2 µm. The comparison of the unpolished and polished acrylic resin specimens showed a reduction in the surface roughness of up to 75%, from 0.8 µm to 0.2 µm. The comparison of unpolished and polished valplast specimens showed a reduction in the surface roughness of only up to 33.3%, from 0.9 µm to 0.6 µm. The colony forming unit (CFU) value was obtained for each immersed group, as previously described in the methods.



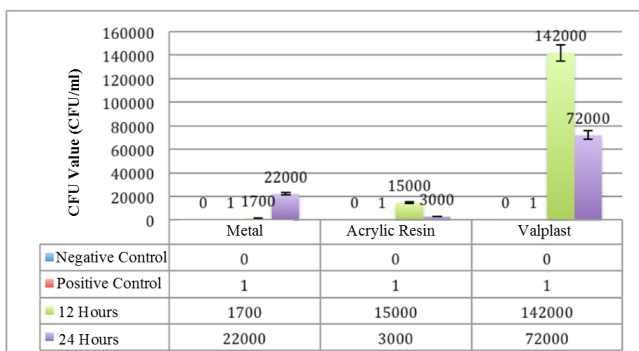
**Figure 1.** CFU levels resulting from the immersion of unpolished and polished specimens in TYS20B liquid media with and without *S. mutans* for 12 hours.

Figure 1 showed the CFU value for the negative control group was zero, while the value for the positive control group was 1 CFU/mL. A difference in the CFU value of up to 70.6% was seen between the unpolished and polished metal after incubation for 12 hours, from  $1.7 \times 10^3$  CFU/ml to  $0.5 \times 10^3$  CFU/ml. A difference of up to 95.3% was seen between the unpolished and polished acrylic resin after incubation for 12 hours, from  $15 \times 10^3$  CFU/ml to  $0.7 \times 10^3$  CFU/ml.



**Figure 2.** CFU levels resulting from the immersion of unpolished and polished specimens in TYS20B liquid media with and without *S. mutans* for 24 hours.

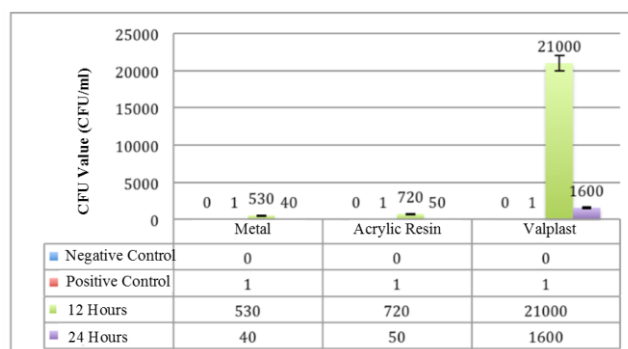
Figure 2 showed the CFU value for the negative control group was zero, while the CFU value for the positive control group was 1 CFU/mL. Furthermore, a difference in the CFU values of the unpolished and polished metal of up to 99.8% was seen after incubation for 24 hours, from  $22 \times 10^3$  CFU/ml to  $0.04 \times 10^3$  CFU/ml. The difference for the acrylic resin specimens was 98.3%, from  $3 \times 10^3$  CFU/ml to  $0.05 \times 10^3$  CFU/ml. The difference for the valplast specimens was 97.8%, from  $72 \times 10^3$  CFU/ml to  $1.6 \times 10^3$  CFU/ml.



**Figure 3.** CFU levels resulting from the immersion of unpolished and polished specimens

in TYS20B liquid media with and without *S. mutans* for 12 hours and 24 hours.

Figure 3 showed the CFU value for the negative control group was zero, while the CFU value for the positive control group was 1 CFU/mL. An increase of up to 1,194.2% was seen in the CFU value of the unpolished metal between 12 and 24 hours of incubation, from  $1.7 \times 10^3$  CFU/ml to  $22 \times 10^3$  CFU/ml. Meanwhile a decrease in the CFU value of up to 80% was seen in the unpolished acrylic resin between 12 and 24 hours, from  $15 \times 10^3$  CFU/ml to  $3 \times 10^3$  CFU/ml. A decrease of 49.3%, from  $142 \times 10^3$  CFU/ml to  $72 \times 10^3$  CFU/ml, was seen in the unpolished valplast between the incubation periods of 12 and 24 hours.



**Figure 4.** CFU levels resulting from the immersion of polished specimens in TYS20B liquid media with and without *S. mutans* for 12 hours and 24 hours

Figure 4 showed the CFU value for the negative control group was zero, while the CFU value for the positive control group was 1 CFU/mL. A reduction was seen in the CFU values for the polished metal, acrylic resin, and valplast between the incubation periods of 12 and 24 hours. For the metal, the difference was 92%, from  $0.5 \times 10^3$  CFU/ml to  $0.04 \times 10^3$  CFU/ml. For the acrylic resin, the difference was 92.9%, from  $0.7 \times 10^3$  CFU/ml to  $0.05 \times 10^3$  CFU/ml. For the valplast, the difference was 92.4%, from  $21 \times 10^3$  CFU/ml to  $1.6 \times 10^3$  CFU/ml. The Ra and CFU levels obtained from the experiments were analyzed using a bivariate Pearson correlation test to measure the strength and direction of the linear relationship of both variables. The Pearson test indicated a strong positive correlation between the polished metal and the *S. mutans* CFU value at 12 hours and 24 hours, with a correlation value of 1 for each group.



Furthermore, a strong positive relationship was seen between the polished acrylic resin and the *S. mutans* CFU values at 12 hours and 24 hours, with correlation of 1 for each group. A strong positive relationship was also seen between the polished valplast and the *S. mutans* CFU values at 12 hours and 24 hours, with correlation of 1 for each group. The growth cycle of *S. mutans* on each denture base material, which are metal, acrylic resin, and valplast are shown in Figure 5, 6, and 7 respectively.

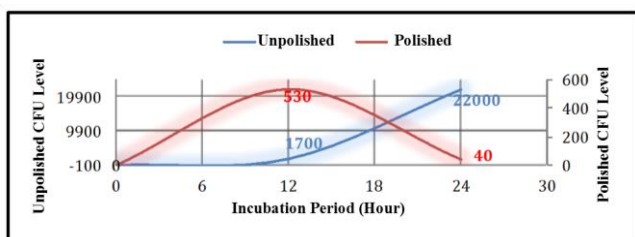


Figure 5. Growth cycle of *S. mutans* on metal.

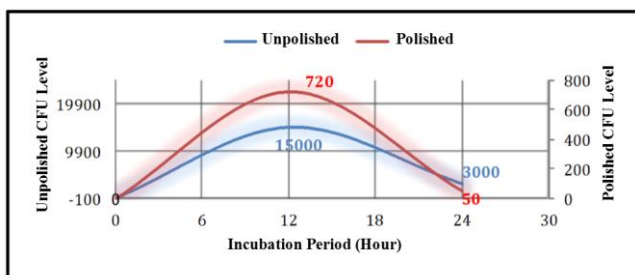


Figure 6. Growth cycle of *S. mutans* on acrylic resin.

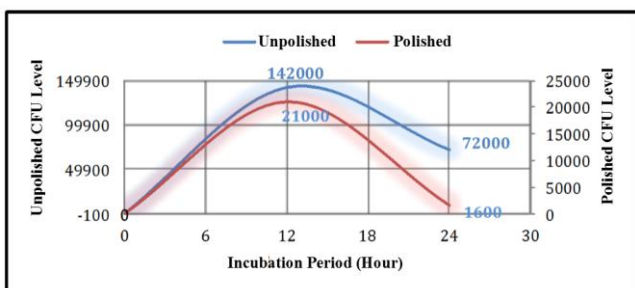


Figure 7. Growth cycle of *S. mutans* on valplast.

## Discussion

The results of the specimen surface roughness tests showed a decrease in the surface roughness due to polishing for every material (metal, acrylic resin, and valplast). These results accord with the research conducted by Sandoval et al., which compared the surface roughness of an alloy of copper and aluminum (Co-Al) before and after polishing. The

study also compared the surface roughness of heat-cured acrylic resin and cold-cured acrylic resin before and after being polished. Sandoval et al.<sup>16</sup> used an atomic force microscope (AFM) to record the roughness value in units of nm, because the radius tip of the stylus was smaller than than the stylus of the surface roughness tester; therefore, with better spatial resolution, the AFM provided measurements with a higher accuracy than the surface roughness tester. The roughness examination with the AFM also showed a reduction of the surface roughness of a Co-Cr alloy after it was polished from 743.69 nm to 97.47 nm (0.744–0.098  $\mu\text{m}$ ). A reduction of the surface roughness of heat-cured acrylic resin was also found after it was polished from 93.25 nm to 48.30 nm (0.093–0.048  $\mu\text{m}$ ).<sup>16</sup>

The results of this study are consistent with research conducted by Abuzar et al.,<sup>10</sup> which compared the surface roughness of two unpolished and polished polyamide surfaces with PMMA (an acrylic resin). The surface roughness examination was performed using a profilometer (Stylus Profiler XP-2, Ambios Technology, Santa Cruz, CA, USA) with a diamond stylus similar to the surface roughness tester used in this study. The surface roughness was recorded in units of  $\mu\text{m}$ . The profilometer showed that a polyamide denture base is 7 times finer after being polished with a conventional technique, while acrylic resin is 20 times finer after being polished with the same technique.<sup>10</sup>

The growth rate of *S. mutans* on unpolished metal was faster than on polished metal, as seen on Figure 5. The difference was due to the surface roughness of the unpolished metal, with a value of up to 0.6  $\mu\text{m}$  ( $R_a > 0.2 \mu\text{m}$ ). The rough surface facilitated the adhesion of *S. mutans* to unpolished metal. Furthermore, a reduction of the *S. mutans* CFU level on polished metal was seen after 12 hours. This reduction occurred because the polished metal had a smooth surface with a surface roughness value of 0.2  $\mu\text{m}$  ( $R_a = 0.2 \mu\text{m}$ ), making it difficult for *S. mutans* to adhere to the surface. In contrast, on the unpolished metal, an increase in the *S. mutans* CFU level was seen after 12 hours. This may have been because the surface roughness of the unpolished metal was above the clinically accepted threshold, facilitating the adhesion of *S. mutans* to the surface. Therefore, *S. mutans* was still growing after 12 hours on unpolished metal.<sup>1,9,17,18</sup>

The growth rate of *S. mutans* on unpolished acrylic resin was faster than on polished acrylic resin, as seen in Figure 6. The difference was due to the rough surface of the unpolished acrylic resin, with a value of up to 0.8  $\mu\text{m}$  ( $R_a > 0.2 \mu\text{m}$ ). Furthermore, the microporosity of unpolished acrylic resin is higher than that of polished acrylic resin. The rough surface and the microporosity facilitate the adhesion of *S. mutans* to unpolished acrylic resin.<sup>16</sup> A reduction in the *S. mutans* CFU level on unpolished and polished acrylic resin after 12 hours can be seen in Figure 6. This reduction may have occurred due to the release of an acrylic resin monomer residue. The residual monomer is toxic for living organisms, so it could inhibit the growth and adhesion of *S. mutans*.<sup>19</sup> The reduction in the *S. mutans* CFU level after 12 hours accords with the research conducted by Kunze et al.<sup>14</sup> that showed that biofilm cells achieved their maximum abundance after 8–12 hours as a result of the separation of biofilm fragments from the mature biofilm.

The growth rate of *S. mutans* on unpolished valplast was faster than on polished valplast, as seen on Figure 8. The difference was due to the higher surface roughness of unpolished valplast (up to 0.9  $\mu\text{m}$ ;  $R_a > 0.2 \mu\text{m}$ ) compared to that of polished valplast (0.6  $\mu\text{m}$ ;  $R_a > 0.2 \mu\text{m}$ ). The rough surface facilitated the adhesion of *S. mutans*.<sup>1,9</sup> Furthermore, a reduction in the *S. mutans* CFU level after 12 hours was seen. This reduction occurred because valplast is a nonporous material, so it has little surface area for *S. mutans* to adhere to.<sup>20</sup> This result accords with the theory of biofilm formation of Suwandi et al.<sup>21</sup>, who reported that there are three phases of biofilm formation: the adhesion phase (0–4 hours), the active accumulation phase (4–20 hours), and the maturation phase (after 20 hours). In this study, the high CFU level seen in the 12-hour incubation period was due to the active accumulation phase of the biofilm formation. The increase in the quantity of biofilm cells was due to the accumulation and rapid growth of the bacteria in the active accumulation phase.<sup>22</sup> Meanwhile, the reduction in the CFU level after 24 hours of incubation was due to the maturation phase, during which the growth of the bacteria slowed or stopped.<sup>14,21</sup> There was a strong positive correlation between the polishing of each material and the *S. mutans* CFU level at the 12-hour and 24-hour incubation periods. This result

accords with those of Pachava et al.<sup>1</sup>, Hashiguci et al.<sup>9</sup>, Al-Kheraif<sup>17</sup>, and Gharechahi et al.<sup>18</sup>, who explained that a reduction in the surface roughness value could reduce biofilm retention and accumulation.

## Conclusion

The reduction in the surface roughness value after the polishing of metal, acrylic resin, and valplast denture base material will be followed by a reduction in the CFU levels of *S. mutans*. Further research should be conducted on the effects of the surface roughness of metal, acrylic resin, and valplast denture base material on *S. mutans* using a variety of incubation periods, different types of oral *Streptococcus*, and different conditions of the breeding media. Field tests of these effects in the oral mucosa are also necessary. Further research of clinical importance is also needed regarding the proper cleaning methods and materials for metal, acrylic resin, and valplast denture base material.

## Declaration of Interest

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