Evaluation of Candida albicans attachment to flexible denture base material (valplast) and heat cure acrylic resin using different finishing and polishing techniques

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ABSTRACT

Background: Surface roughness of the denture plays a major role in the adhesion of microorganisms, it affected primarily by the finishing and polishing techniques, the aim of this study was to evaluate the effect of two finishing and four polishing techniques on the surface roughness of flexible denture base (Valplast) and heat cure acrylic and its effect on Candida albicans attachment with the effect of saliva on Candidal adhesion.

Material and method: Three hundred twenty square samples (2cm*2cm*3mm) were prepared (160 flexible acrylic and 160 heat cure acrylic as control), the flexible acrylic and heat cure samples were divided into two major groups according to the finishing method. Group A finished by stone bur, tungsten carbide bur and sand paper. Group B finished by special green cone (silicone finisher bur). Each group divided into four subgroups according to the polishing method, subgroup contains (20 samples flexible acrylic, 20 samples heat cure acrylic). The mean surface roughness values (Ra) were gained for each sample after finishing and polishing, the samples then incubated in media containing suspension of Candidal cell (conc. of 1*10^6 cell/ml) for sixty minutes at room temp., after that they were dried, fixed and stained. Ten samples of each subgroup were incubated in artificial saliva for thirty minutes before adherent assay.

Result: Statistical analysis revealed that flexible acrylic samples that were finished by special green cone with the different polishing techniques were smoother in profilometric study with significance difference in Candidal attachment.

Conclusion: Using of special green cone with different polishing techniques for flexible acrylic and heat cure acrylic will yield smoother surface more than using stone bur, tungsten carbide bur and sand paper, Candida albicans attachment affected by the finishing and polishing method, the method that yield less surface roughness values have less Candidal attachment, saliva decreases the attachment of C. albicans.


INTRODUCTION

Thermoplastic resins have been used in dentistry for over 50 years (1). During this time the application of these materials continued to grow and their interest increased by both the profession and public (2).

Valplast is a (thermoplastic material) which is a nylon thermoplastic with unique physical and esthetic properties and excellent retention it can be used whenever denture is indicated for patients provides unlimited design versatility and eliminates the concern about acrylic allergies. Application of thermoplastic resin originally involved flexible tooth born partial dentures. Currently dental applications include: performed partial denture clasp, flexible tooth born partial denture framework, single cast partial dentures, temporary crown bridges, occlusal appliances.

Thermoplastic resin in spite of all these benefits it has some difficulty to adjust and polish. Smooth and polished surfaces of the prosthesis play a major role for the patient comfort and denture longevity (3).

Yeast of the genus Candida are commonly present in the plaque, their adhesion to the surface of the denture may cause Candidosis infections to the patient.

The surface irregularities increase the probability of bacterial accumulation, and Candida adhesion than smooth surface, surface roughness of prostheses providing niches in which the microorganisms are protected from sheer forces and oral hygiene measures (4), the surface of the resins used in the construction of the denture can be finished and polished using variety of techniques, burs sand paper, discs, paste & pumice (5).

In this study an attempt to improve the surface smoothness of flexible acrylic and heat cure acrylic denture base material was done in order to reduce the adhesion of C. albicans that may be attached to it.

MATERIALS AND METHOD

Preparation of PMMA samples

Metal pattern was prepared of dimension (20*20*3 mm), the conventional flasking technique that used for complete denture
Construction was followed (mixing, packing, curing and deflasking).

**Preparation of flexible acrylic samples**

**Mold preparation**

The flask is different from that used for processing heat cure acrylic samples by having four screw (one in each corner) so no need for clamp in this flask as shown in (Figure 1).

<table>
<thead>
<tr>
<th>Material</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Heat cured acrylic resin (powder and liquid)</td>
<td>Vertex, Holland</td>
</tr>
<tr>
<td>2. Valplast plastic bag (flexible acrylic)</td>
<td>FDA, MSDs, ISO, USA</td>
</tr>
<tr>
<td>3. Green Cone Polishing Kit</td>
<td>Vertex, Holland</td>
</tr>
<tr>
<td>4. Sabouraud's dextrose agar</td>
<td>Mumbai-India</td>
</tr>
<tr>
<td>5. Gram Stain Kit</td>
<td>Spain</td>
</tr>
</tbody>
</table>

Position the injection sprues

**Figure 1: Special flask for flexible acrylic**

Using wax stick (4 mm in diameter) was attached to each pattern in the one corner then the four sprues were attached together as one sprue when reaching the orifice in the centre of side part of the flask (Figure 2).

**Figure 2: Wax sprues attached to the metal pattern**

**Embed the top half of the flask**

The top hal of the flask was placed on the bottom half, ensuring complete, intimate contact and closure of the halves secure metal flask brackets to the flask and tightened.

**Boil out**

After setting of the stone, the bolt were loosen, the metal flask brackets were removed, the flask was placed in boiling water, all wax discarded, flushed and cleaned thoroughly (Figure 3).

**Figure 3: The flask after boiling out.**

**Heating cycle**

The flexible acrylic bag or cartridge sleeve was placed in the heating chamber above the flask in the plastic injection assembly and was allowed to heat up about 15 minutes reaching temperature of 285° C according to the manufacturer instruction.

After that the injection started by sliding the shaft of the plastic injection machine until the spring of the machine is compressed completely, (Figure 4 and 5). After that the flask was allowed to cool and deflaking was done.

**Figure 4: Injection machine**

**Figure 5: Injection process**
Finishing
The samples were divided into two major groups:
**Group A: 160 samples (80 samples flexible acrylic and 80 samples heat cure acrylic)**
Finished according to the method suggested by Ulusoy 1986:
- **Stone** for two minute with low speed 1500 rpm and low pressure.
- **Tungsten carbide bur** for two minute with low speed 1500 rpm and low pressure.
- **Sand paper** for 1 minute with low speed 1500 rpm

**Group B: 160 samples (80 samples flexible acrylic and 80 samples heat cure acrylic)**
Finished by special green finishing cone (silicone bur) for two minutes, 5000 rpm, low pressure)
The samples are finished using special finishing machine designed for this study (Figure 6).
This machine has capability of movement of the sample in three directions on a fixed path for each direction (horizontal, vertical and axial).

Figure 6: Finishing machine

To standardize the polishing procedure, the samples were held in a constant distance from the rotating rag wheel of the lath machine on especial stand each sample was placed in an ultrasonic bath for five minutes and dried using a high-pressure air hose prior to measuring surface roughness.

Surface roughness test
The surface of the test specimen was analyzed with surface roughness tester profilometer to study the effects of finishing and polishing agent on the mircogometry of the test surface., two readings were recorded for each specimen and the mean value for each specimen was the average of two readings

Examination of the samples
Some of the samples were examined under special Optical Microscope (Nikon Eclipse, with Nikon digital camera DXM1200F)

Isolation of *Candida albicans*
The *C. albicans* organism was isolated from culture obtained from patients with denture stomatitis, swab has been taken from the palatal mucosa of the patient, using cotton tipped swab the swab was cultured on sabourauds dextrose agar and incubated at 37°C for 72 hr and then kept at 4°C for further investigation.

Preparation of sabouraud’s dextrose agar
Preparation of the agar was done according to the manufacturer instructions, the media was sterilized in autoclave at 15 b,121°C for 15 minutes, the culture media was cooled.

Identification of *C. albicans*

A. Colony morphology
Colonies of *C. albicans* appear smooth creamy in color with a yeast odor and typically medium size (1.5-2) mm diameter which later develop into high convex, off-white larger colonies after 2 days, (Figure 7).

Microscopical examination
under light microscope. Candida appeared as gram-positive small oval or budding yeast cells. (Figure 8).

C. Germ tube formation
the presence of germ tube. On incubated candida in human serum is characteristic of *C. albicans*.

D. Biochemical Identification
The API –Candida system was used for the identification of fungal isolate, this system consist of 10 different biochemical test.
Preparation of *C. albicans* suspension

a- preparation of the peptone broth was done according to manufacturer instructions, the broth was transferred into tubes small inoculums from isolated yeast colonies were suspended in the broth, there after the tubes were incubated at 37°C for 24 hours.

b-Preparation of the recommended concentration of *C. albicans* suspension:-

Gradual dilution of the broth was done by addition of normal saliva and monitoring the concentration of *C. albicans* until reading the exact concentration needed for the study (1*10⁶ cell/ml).

Adherence assay

Twenty samples of each sample of the denture base material were deposit in 20 ml of yeast suspension sterile petridishes, ten samples of each subgroup were incubated for 60 minutes at room temp., then the acrylic sample were washed twice by gentle agitation in normal saline for one minute, after they were dried, adherent yeast were fixed with methanol and stained with crystal violet for one minute, ten samples were incubated in artificial saliva at room temperature for 30 minutes and the adhesion assay continued as previously described.

Enumeration of adherent Candida

Adherent yeast cells in 15 field of view (0.4 mm² / field) at * 400 magnification power were enumerated and mean number for the 15 field then gained.

**RESULTS**

Effect of finishing and polishing techniques on the surface roughness of acrylic specimens, for flexible acrylic samples and heat cure acrylic samples finished by (A) and polished by the four polishing technique table (2), show high significant difference between flexible and heat cure acrylic finished ad polished by the same method at level (p<0.001)., for flexible acrylic samples and heat cure acrylic samples finished by the special green cone bur(B)and polished by the four polishing technique table (3), show high significant difference between flexible and heat cure acrylic finished and polished by the same method at level (p<0.001).

<table>
<thead>
<tr>
<th>Polishing Types</th>
<th>(F a.) Mean</th>
<th>(H a.) Mean</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>3.63</td>
<td>1.86</td>
<td>12.90</td>
<td>0.000 ***</td>
</tr>
<tr>
<td>A2</td>
<td>3.86</td>
<td>1.39</td>
<td>15.11</td>
<td>0.000 ***</td>
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<tr>
<td>A3</td>
<td>1.98</td>
<td>1.31</td>
<td>5.70</td>
<td>0.000 ***</td>
</tr>
<tr>
<td>A4</td>
<td>1.79</td>
<td>1.36</td>
<td>3.32</td>
<td>0.000 ***</td>
</tr>
</tbody>
</table>

Table 3: The difference between flexible and heat cure acrylic in (B) finishing method in µm

<table>
<thead>
<tr>
<th>Polishing types</th>
<th>(F a.) Mean</th>
<th>(H a.) Mean</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>0.92</td>
<td>0.82</td>
<td>1.81</td>
<td>0.08 (NS)</td>
</tr>
<tr>
<td>B2</td>
<td>0.64</td>
<td>0.43</td>
<td>6.56</td>
<td>0.000 ***</td>
</tr>
<tr>
<td>B3</td>
<td>0.67</td>
<td>0.45</td>
<td>6.44</td>
<td>0.000 ***</td>
</tr>
<tr>
<td>B4</td>
<td>0.63</td>
<td>0.47</td>
<td>4.07</td>
<td>0.000 ***</td>
</tr>
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</table>

Effect of finishing & polishing technique on the candidal attachment

For the flexible acrylic and heat cure acrylic samples finished by(A and B technique), heat cure shows less Candidal adhesion than flexible acrylic
samples with high significant difference at level (p<0.001) table (4).

**Table 4: t-test between Candidal adhesion to the surface of flexible & heat cure (conc. 1*10^6 cell/ml)**

<table>
<thead>
<tr>
<th>Polishing types</th>
<th>Mean (F a.)</th>
<th>Mean (H a.)</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>19.60</td>
<td>10.00</td>
<td>8.42</td>
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<tr>
<td>A2</td>
<td>16.99</td>
<td>7.25</td>
<td>9.21</td>
<td>0.000 ***</td>
</tr>
<tr>
<td>A3</td>
<td>10.46</td>
<td>5.24</td>
<td>6.96</td>
<td>0.000 ***</td>
</tr>
<tr>
<td>A4</td>
<td>6.17</td>
<td>5.14</td>
<td>1.73</td>
<td>0.10 (NS)</td>
</tr>
<tr>
<td>B1</td>
<td>9.44</td>
<td>6.80</td>
<td>5.75</td>
<td>0.000 ***</td>
</tr>
<tr>
<td>B2</td>
<td>6.65</td>
<td>5.86</td>
<td>3.03</td>
<td>0.01 **</td>
</tr>
<tr>
<td>B3</td>
<td>7.38</td>
<td>6.36</td>
<td>3.76</td>
<td>0.01 **</td>
</tr>
<tr>
<td>B4</td>
<td>7.10</td>
<td>5.80</td>
<td>5.22</td>
<td>0.000 ***</td>
</tr>
</tbody>
</table>

Effect of saliva on the adhesion of C. albicans to samples statistical analysis shows that saliva decrease in the adhesion of the C. albicans to the surface of flexible & heat cure acrylic samples (Fig.10).

**Figure 9: Bar chart for the effect of saliva on Candidal adhesion**

**DISCUSSION**

Effect of Finishing & Polishing Technique on the Surface Polyamides have been reported as being difficult to finish and polish due to their low melting temperature, Fraying at the margins of the polyamide specimens was noticed occasionally during polishing of the samples in this study which may have occurred due to overheating of the surface and exposure of fibers. Furthermore the rate of cooling of processed polyamide affects the surface properties and it has been mentioned that very slow cooling produces a strong and relatively stiff material but also a rough surface with special green cone the use of green cone, progressively improving the surface smoothness, because it promotes surface abrasion with material removed generating traces or notches with progressively of lower dimension as finer grits are utilized.

Finishing with silicone finisher produced a surface that was smeared possibly because of over heating of the rubber phase that is incorporated into the polyethylmethacrylate, although the direction of the silicone point was moved around the entire surface of the specimen, considerable heat could be generated at the specimen -finisher polisher interface.

Pre polishing Rubberizing by rubber bur or disc may promote removing of course grooves and notches in the surface of acrylic this effect appear to have more print on the flexible acrylic samples that heat care samples that may be due to the special nature of the material itself as the flexible acrylic material which is a thermoplastic resin mainly Po lyamide (Nylon) which is more wear resistance than heat care acrylic (Polymethylmethacrylate)

**Effect of Finishing and Polishing Technique on Candida Albicans Attachment**

The result of the microbiological study have showed that the finishing and polishing technique that show lower surface roughness value have less number of Candidal cells attachment . This can be explained that materials with the roughest surface may serve as reservoir, with surface irregularities providing an increase microorganism retention and protection from shear forces., rough surface has irregularities inducing adhesion of Candida and bacteria, these superficial defect such as voids and micro cracks on surface were possible sites for Candidal adhesion.

**Effect of Saliva on the Candida Albicans Attachment to The Flexible and Heat Cure Samples**

The presence of saliva decrease Candidal attachment this may be explained that, also the pellicle of the Saliva could act as blocker of a non specific adhesion of the yeast to the surface of the acrylic, so Saliva significantly reduce the adherence of C. albicans to the surface in vitro, another explanation is that thin biofilm of the acquired salivary pellicle can significantly reduce free energy on hard intraoral surfaces that may affect Candidal adhesion.

**REFERENCES**


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