THE EFFECT OF CURING CYCLE OF DENTURE BASE (PMMA) ON MICROCOLONIZATION OF CANDIDA ALBICANS

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ABSTRACT

The purpose of the study was to assess the growth of Candida Albicans on Polymethylmethacrylate (PMMA) as a denture base, using various curing cycles.

Total 200 samples of denture bases were prepared divided into five categories, cut into 2×2 cm, with the thickness of 1.0 mm for the analysis. 160 out of them were further divided into 4 groups for heat cure water bath polymerization. They were assessed on different temperatures i.e. 45°C, 100°C, 70°C for the time span of 45, 60, 420 and 540 min respectively. The specimens of the 5th group were cured chemically. Every specimen of all the five groups was kept in the bottles along with the food stuff for 12, 24, 168, and 336 hrs. The specimens were then mounted on the glass slides to examine under the light microscope for the analysis of Candida Albicans.

Results showed that the growth of Candida Albicans observed to be reduced in heat cure polymerization as compared to the ones, cured chemically. Thus concluded that among all the sample types, the group D cured at the temperature of 70°C for 540 min have shown the minimum growth of Candida Albicans.

Key word: Polymethylmethacrylate (PMMA), Curing Cycles, Candida Albicans

INTRODUCTION

A complete denture is a removable dental prosthesis, that replaces the entire dentition and associated structure of maxilla and mandible.1 Acrylic plastics have been extensively manufactured for artificial denture teeth.2 Acrylic resin is available in powder and liquid form, mixing reaction is called Polymerization.3 It can be achieved by heat activation (water bath or microwave- energy), chemical activation and light application.4 According to Saavedra G et al., (2004), water bath polymerization is the most widely used method for denture processing.5 There are various unhealthy effects on the oral mucosa by inadequate oral hygiene resulting in the built up of denture plaque containing Candida.6 The fungus Candida Albicans is commonly associated with the denture stomatitis, common in elderly females as compared to male.7

Daniluk T et al., (2006) observed that denture Stomatitis was associated mainly with Candida Albicans infections.8 The results of Barbeau J, et al., (2003) showed that denture-related stomatitis, revealed the presence of yeast on the denture, linked with the inflammation, and favours the colonization of Candida.9 Mizugai H, et al., (2007) concluded that the occurrence of multiple Candida species sets denture wearers apart from subjects who do not wear dentures.10

According to a prior study, Candida Albicans adheres with oral bacteria to Methylmethacrylate den-
ture base, and provide a reservoir of organisms, and infect the mucosa.\textsuperscript{11}

The purpose of this study is to evaluate the effect of processing cycle on denture base for the growth of Candida colonies in complete denture, and to understand the possibility for the reduction of this growth, which could be helpful for the health of oral mucosa and resulting into different types of pathologies.

**METHODOLGY**

A total of 200 specimens measuring 2 x 2 cm with 1 mm thickness, were prepared with acrylic resins using the same brand (STELLON, Standard denture base acrylic Powder / Liquid, Heat Cure. Karachi Dental). Modelling wax (SUPERIOR® Modelling Wax) was used for preparing the specimens. 1 mm thickness of the wax sheet was helpful to create the required space to craft the specimen of 1 mm thickness. The waxed-up models were invested in Plaster of Paris (National Plaster of Paris, Thatta Cement Co. Ltd.) using two mould Brass flask. Dewaxing was carried out, heating it for 30 minutes at 60°C using (Electrical Thermostatic Water Baths (2L4H) Anjum MC028110202 Made in China.). Separating medium (Shiva Products, Thane, Maharashtra, India) was applied after de waxing according to the manufacturer’s instructions. The mixed acrylic resin was packed in the flask at doughy stage, and cured according to the routine procedure.

Various methods have been used for curing the acrylic resin i.e. by heat activation (water bath or microwave-energy), chemical activation and light application.\textsuperscript{4}

Heat and chemical activation methods have been selected to be used in this study. The specimens were divided into five groups designated as A, B, C, D and E. The specimens in group A were cured at 45°C for 45 minutes.

The specimens in group B, C and D were cured at 100°C, 70°C and 70°C for 60 min, 420 min and 540 min. respectively.

The specimens in group ‘E’ were self cured at room temperature, using (SIMPLEX, Denture repair, Powder / Liquid, Self Cure. Karachi Dental).

Finishing and trimming of the specimens was carried out. Polishing was done by using pumice slurry with cotton buff.

All the specimens of group A -1, A -2, A -3, and A -4, having 10 samples each were kept in the sterilized bottles tagged with code numbers of individual sample with the food stuff for 12, 24, 168 and 336 hours. Similarly rest of the samples i.e. B -1, B -2, B -3, B -4, C -1, C -2, C -3, C -4, D -1, D -2, D -3, D -4, E -1, E -2, E -3, and E -4, were treated in the same way. Having done this treatment, the samples were removed from the bottles with the help of a tweezers (Dental examination instrument). Samples of the heat cure acrylic resin were placed in incubator for 72 hrs at 37°C and subcultured it on sabouroude agar plate for the isolation and fungal growth & blood agar Macconkey’s agar for bacterial growth, there are many type of fungal or bacterial growth observed after 24 hours.

Cold cure samples were transferred to brian heat infusion broth bottles and then observed the turbidity after 48 hours. Inoculation was done with the drop of broth on the blood agar and Mac Conkey’s agar plate for the isolation of bacterial growth after 24 hours of incubation. It was observed that growths were present on plates i.e. G-ve and G+ve. The specimens were then examined for Candida Albicans under Light Microscope with Olympus B x51, Camera.

Magnification × 400. The actual count of colonies for bacteria, thus obtained were expressed by calculating with formula of colony-forming units CFU.\textsuperscript{12}

\[
\text{CFU} = \frac{\text{Bacterial Colony Count}}{\text{Volume used} \times \text{Reciprocal of dilution}}
\]

The data was collected and statistical analysis was done on computer software using SPSS version 17.

**RESULTS**

Total number of 200 specimens, measuring 2 x 2 cm with 1 mm thickness, were prepared with acrylic resins, divided into five groups, four of them processed by heat activation and the specimens of the fifth group was processed by chemical activation. After performing the whole procedure as in the above mentioned methodology, the specimens were examined under Light Microscope for estimation for the count of Candida Albicans colonies.

The count of colonies for bacteria were estimated and expressed using the formula of colony-forming units CFU .12
As each sample was composed of 40 specimens, so the data of all the 200 specimens was collected and calculated by the above mentioned formula. It was analysed by SPSS version 17 where the P-value being 0.05. The results of the analysis showed that the P-value between the group A and B is 0.594, in the group A and C is 0.994, in the group A and D is 0.996, between the group A and E is 0.985, between the group B and C is 0.548, between the group B and D is 0.728, in the group B and E is 0.536, between the group C and D is 0.979, in the group C and E is 0.988 and between the group D and E is 0.986.

All the above mentioned results showed insignificant values i.e. >0.05, comparing, all the groups with each other.

On the other side the % age of the colony counts was also calculated and an average % age was taken and considered for understanding the apparent results, which revealed that the average % age of Candida Albicans colonies count in group “A” is 5.75 %, in group “B” is 3.00 %, in group “C” is 5.5 %, in group “D” is 2.5 %, and in group “E” is 46.25 %. These results appeared showing the differences in the colony counts in all the groups. It was thus revealed that the minimum colony count was seen in the procedures adopted in the group “D” i.e. in the heat curing procedures done under the temperature of 70°C for 540 minutes.

So it was understood that the procedure done under such protocol would be the most appropriate to be adopted.

### TABLE 1: THE EFFECT OF HEAT CURE CYCLES OF ACRYLIC RESIN DENTURE BASE (PMMA) ON MICRO-COLONIZATION OF CANDIDA ALBICANS

<table>
<thead>
<tr>
<th>Groups PMMA</th>
<th>No of samples</th>
<th>Curing Cycle</th>
<th>Period of Acrylic Resin Analysis</th>
<th>Candida</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Temperature</td>
<td>Time in food stuff</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>A-1</td>
<td>10</td>
<td>45°C</td>
<td>45 min</td>
<td>5%</td>
<td>5.75%</td>
</tr>
<tr>
<td>A-2</td>
<td>10</td>
<td></td>
<td>24 hours</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>A-3</td>
<td>10</td>
<td></td>
<td>168 hours</td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>A-4</td>
<td>10</td>
<td></td>
<td>336 hours</td>
<td>7%</td>
<td></td>
</tr>
<tr>
<td>B-1</td>
<td>10</td>
<td>100°C</td>
<td>60 min</td>
<td>1%</td>
<td>3.00%</td>
</tr>
<tr>
<td>B-2</td>
<td>10</td>
<td></td>
<td>24 hours</td>
<td>1%</td>
<td></td>
</tr>
<tr>
<td>B-3</td>
<td>10</td>
<td></td>
<td>168 hours</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>B-4</td>
<td>10</td>
<td></td>
<td>336 hours</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>C-1</td>
<td>10</td>
<td>70°C</td>
<td>420 min</td>
<td>5%</td>
<td>5.5%</td>
</tr>
<tr>
<td>C-2</td>
<td>10</td>
<td></td>
<td>24 hours</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>C-3</td>
<td>10</td>
<td></td>
<td>168 hours</td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>C-4</td>
<td>10</td>
<td></td>
<td>336 hours</td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>D-1</td>
<td>10</td>
<td>70°C</td>
<td>540 min</td>
<td>2%</td>
<td>2.5%</td>
</tr>
<tr>
<td>D-2</td>
<td>10</td>
<td></td>
<td>24 hours</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>D-3</td>
<td>10</td>
<td></td>
<td>168 hours</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>D-4</td>
<td>10</td>
<td></td>
<td>336 hours</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>E-1</td>
<td>10</td>
<td>Autopolymerzation</td>
<td>12 hours</td>
<td>40%</td>
<td>46.25%</td>
</tr>
<tr>
<td>E-2</td>
<td>10</td>
<td></td>
<td>24 hours</td>
<td>45%</td>
<td></td>
</tr>
<tr>
<td>E-3</td>
<td>10</td>
<td></td>
<td>168 hours</td>
<td>50%</td>
<td></td>
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<tr>
<td>E-4</td>
<td>10</td>
<td></td>
<td>336 hours</td>
<td>50%</td>
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</table>
DISCUSSION

Oral mucosa is a very sensitive tissue and effected by various external stimuli and by inadequate oral hygiene resulting into an unhealthy conditions like denture stomatitis built up by denture plaque containing *Candida Albicans*.6,7

The colonization of *Candida Albicans* on the oral mucosa and on the surface of dentures may be the first step leading to an infection.13

Number of patients in our clinics also, seen reporting about denture associated problems of oral mucosa, which remains to be a critical issue and needs to be understood and solved. In this study, the effort was done to explore the different causative factors regarding the preparation of the dentures in the laboratory. Rough surfaces can cause discoloration of the prosthesis, may contribute to microbial colonization.14

According to a prior study long cycle increased, and water-bath post-polymerization reduced the cytotoxicity of Lucitone 550 processed by long cycle.15 Another report of the study concluded that water bath post-polymerization heat treatment at 55°C for 60 min improved the biocompatibility as compared to the microwave post-polymerization.16

In this study polymerization was done at a temperature of 70°C 540 min, which revealed that more polymerization time was required for achieving better results. The reason for the longer duration of time in this study could be the functioning of the thermostat of the equipment and the brand of the acrylic resin.

C.-P. Lai *et al.*, (2003) compared water bath polymerization with microwave-cured specimens, concluding that the porosity increased in microwave-cured specimens. In this study water bath polymerization was compared with cold cure polymerization and the results showed that the samples prepared by heat curing had a better surface texture for not retaining the colonization of *Candida Albican*.17

It can be be concluded that water bath polymerization had given the better results in both the studies and seemed to be in accordance with each other.

In-Ho Kang *et al.*, (2002) worked on curing cycles of 70°C 100°C for 90 min, 100°C for 20 min and 70°C for 24 hrs, concluding that the long curing cycles showed least shrinkage and found it favourable.18

In this study the curing cycles with temperature of 70°C for 540 minutes have given a better result of finally prepared prosthesis. So a favourable results were obtained in the procedures done in a shorter time span. The results of the present study revealed that the heat cured acrylic resins have given the better results as compared to the preparations by autopolymerization. Such results have also been supported by the prior studies.15,16,17,18

It is important to highlight that, in the heat curing procedures done under the temperature of 70°C for 540 minutes have given the best results, which will ultimately be providing a good prosthesis leading to the reduction in the retention *Candida Albicans*.

CONCLUSIONS

Based upon the result of heat cure and cold cure acrylic resin polymerization and within the limitations of this in vitro study, following conclusions were drawn:

1. Prosthesis prepared by autopolymerization (cold cure) acrylic resin appeared with the increased growth of *Candida Albicans colonies*.

2. Prosthesis prepared by heat cure acrylic resin polymerization procedures done under the temperature of 70°C for 540 minutes have shown the least retention and colonization of *Candida Albicans*.

It is thus be understood that the good knowledge for curing cycle techniques, is very imperative, if not, it would result into poorly prepared porosities leading to the retention *Candida Albicans colonies*, ultimately effecting the health of oral mucosa.

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REFERENCES


The effect of curing cycle of denture base (PMMA) on microcolonization


