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"Comparative Evaluation Of Biofilm Development Of Candida Albicans On Abraded Surfaces Of Heat Cure PMMA And Flexi Denture Material":An In Vitro Study

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Abstract:
Purpose*: Study was to compare the biofilm development of candida albicans on two different types of abraded surfaces of heat cure PMMA and flexi denture material.*
Materials And Methods: *4 blocks of each heat cure resin and flexible denture material were prepared of 10*10*10mm and 10*10*3mm in size respectively. All blocks were then subjected to abrasion with p100 grit size dry sand paper or with denture cleansing brush. One colony of Candida albicans GDH 2346 (NCYC 1467) cultured on Sabouraud dextrose agar (SBA) for 48 hrs and was used to inoculate 10 mL of Peptone water and sugar broth with samples at 37°C for 24hrs. Followed by which gram staining was performed and semi-quantification was done after inoculating on SBA for 18 hours at 37ºC.*
Results: *The highest mean colony forming unit was recorded in heat cure PMMA, and lowest mean colony forming unit was recorded in flexible denture material.*
Conclusion: *Flexible denture material has lesser biofilm development and candidal count as compared to heat cure acrylic resin.*
Keywords: *Heat cure PMMA, Flexible denture material, Biofilm, Candida albicans*

I. Introduction

Candida albicans is a known etiologic agent of chronic erythematous candidosis (denture stomatitis). This inflammatory disorder affects approximately 60% of denture wearers and causes inflammation of oral mucosa in close contact with the denture. As with natural dentition, dentures provide hard nonshedding surfaces that enable the build-up of plaque biofilms over time. Candida biofilm development on denture acrylic resin begins with adhesion, which can either occur directly to the conditioned surface or via a layer of pre-existing denture plaque.[1] 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. The surface of the resins used in the construction of the prosthesis can be finished and polished using variety of techniques.[2]

Polymethyl methacrylate (PMMA) has been the most popular material used for denture fabrication since its introduction in 1937. It has several advantages such as an excellent esthetic characteristic, low water sorption and solubility, adequate strength, low toxicity, easy repair, and a simple molding processing technique. Nonetheless, it has some problems such as polymerization shrinkage, weak flexural, lower impact strength. In recent years, nylon polymer has attracted attention as a denture base material. Polyamide resin was proposed as a denture base material. Nylon is a generic name for certain types of thermoplastic polymers belonging to the class known as polyamides. Nylon is a crystalline polymer, whereas PMMA is amorphous and low fatigue resistance. On the other side, it is reported that this material has several problems such as water sorption, surface roughness, bacterial contamination, warpage, color deterioration, and difficulty in polishing.[3] Valplast (flexible denture material) is a nylon thermoplastic material with unique physical and esthetic properties and excellent retention. Thermoplastic resin in spite of all these benefits has some difficulty to adjust and polish.[4]

The purpose of this study was to compare the biofilm development of *Candida albicans* on two different types of abraded surfaces of heat cure PMMA and flexi denture material. The null hypothesis was that there would not be any difference in biofilm development of *Candida albicans* on abraded surfaces of heat cure resin and flexi denture material.

II. Materials and methods

4 blocks of each heat cure PMMA and flexible denture material were prepared of 10*10*10mm and 10*10*3mm in size respectively (Fig.1). A block was first made in modelling wax of exact dimensions mentioned above and then other 3 blocks were duplicated using the elastomeric rubber based material index of the first disc.

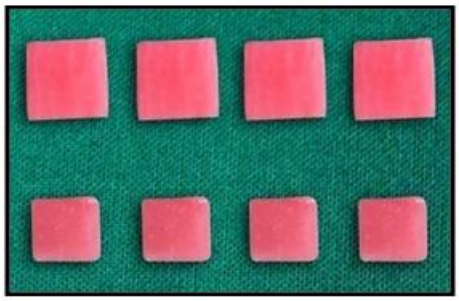

Figure 1. Showing the shape and size of specimens

For Heat cure resin, all 4 blocks were invested in dental plaster and packed using compression moulding technique with heat cure PMMA (MELIODENT). The conventional technique for flasking curing and deflasking was followed. For Flexible denture material, 4 specimen were prepared using injection moulding technique.

All blocks were then subjected to abrasion. 2 blocks of each heat cure resin and flexible denture material were abraded using Sand paper. These specimens were abraded manually with p100 grit size dry sand paper with 10 strokes in one direction parallel to the edge of a ruler, and Other 2 blocks of each material were abraded with denture cleansing Brush **(**CLANDEN**),** 400 strokes in one direction and another 400 strokes in opposite direction. All these abrasion procedure is done by one person. After abrading all the blocks, blocks were sterilsed with microwave (600 watt) for 6 mins. Heat cured resin blocks abraded with sandpaper was named - **HS**, and the ones abraded with brush was named as **HB.** Sameway, for flexible denture material **FS** and **FB** was named(Fig.2).

Figure 2. Naming for each specimen

Microbiological testing

One colony of Candida albicans GDH 2346 (NCYC 1467) cultured on Sabouraud dextrose agar (SBA) for 48 hrs and was used to inoculate 10 mL of Peptone water and sugar broth with samples at 37°C for 24hrs. Test tube named HB contained both HB1 and HB2 samples and HS contained HS1 and HS2. Sameway for flexible denture material samples. After 24 hours samples were checked for biofilm production by taking swabs and performing Gram staining. For semi-quantitation swabs were taken and inoculated on fresh sterile SBA and incubated at 37°C (Fig.3)

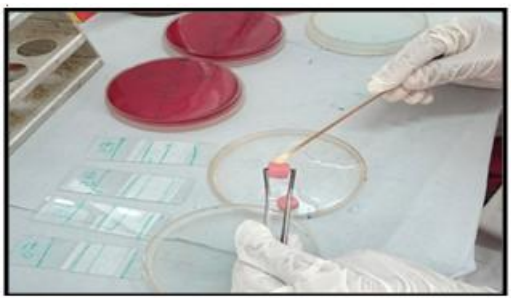

Figure 3. Inoculation of swabs.

They were incubated for 18 hours and then the number of colonies were counted which is then multiplied by 100 to get CFU/ml (Fig.4)

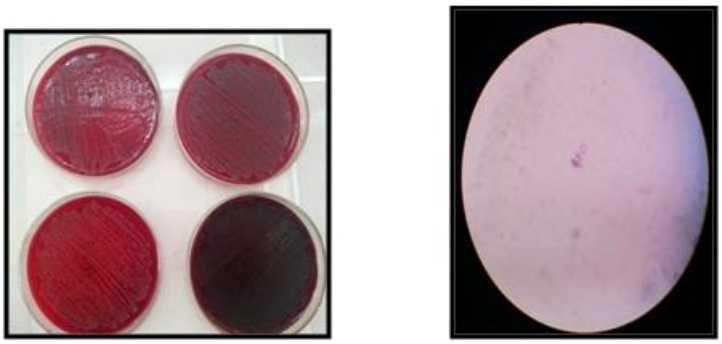

Figure 4. Incubated colonies.

Data were presented as mean and standard deviation (SD) values. Unpaired T-Test (Non-parametric) was used for comparison between mean values.

III. Results

Candidal count of all the samples is shown in Table 1. Results showed that highest mean colony forming unit was recorded in heat cure acrylic resin, 435000 CFU/ml, and lowest mean colony forming unit was recorded in flexible denture material, 175500 CFU/ml (Table 2). Comparing the two material, even the sand paper (rougher) abraded flexible denture material has lesser candidal count then brush abraded (smoother) heat cure acrylic resin. The surface roughness play a major role in microbiological attachment, more rougher the surface more the bacterial count.

Table 1. Colony count of the specimens

SAMPLES	**CANDIDAL COUNT (CFU/ml Unit)**
Heat cure sand paper abraded(HS)	560000 550000
Heat cure brush abraded(HB)	320000 310000
Flexible denture sand paper abraded(FS)	240000 210000
Flexible denture brush abraded(FB)	110000 150000

Table 2. Mean ±SD values of Colony count of specimens.

Sl. No.	Materials	Mean	Standard Deviation	P value
1	Heat cured	435000	11860.35	0.021
2	Flexi	177500	58523.45	

IV. Discussion

Adhesion of microorganisms, especially yeasts, to the denture base materials is an important issue that compromises its service and efficacy.[3] Rougher surfaces contribute to microbial colonization and biofilm formation. Bacterial and fungal species have more of a propensity to adhere to rough denture base materials.one study has suggested that, the conventional polishing technique used for PMMA provided a polyamide surface roughness below the accepted threshold Ra value. Roughness threshold for PMMA and polyamide was considered appropriate to accept the threshold Ra = 0.2 µm in order to explain the results of this study.[5]

The results of this study supports rejecting the null hypothesis that the there is no statistically significant difference in biofilm development of *Candida albicans* on abraded surfaces of heat cure resin and flexi denture material. Flexible denture material has less biofilm development even on rough surface (sand paper) as compared to smoother surface (i.e.brush abraded) of heat cure acrylic resin. Candida growth on the polyamide resin was lower than that observed on the PMMA material. The surface roughness play a major role in microbiological attachment, more rougher the surface more the candidal count.[7] Surface roughness and surface free energy (SFE) may contribute to the positively correlated rate of microbial colonization and plaque maturation on surfaces.[8]

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.[9]

V. Conclusion

Within the limitations of this in vitro study, the following conclusions were drawn:

1. Flexible denture material has lesser biofilm development and candidal count as compared to heat cure acrylic resin, and
2. Specimen that has lower surface roughness value have less number of Candidal cells attachment

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