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Evaluation of *Candida albicans* adherence to different denture base materials

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ABSTRACT

Although, polymethyl methacrylate (PMMA) resin has been the most commonly used material for denture bases, PMMA suffers from major drawbacks. To overcome PMMA resin problems, a new injection modeling thermoplastic resin has been developed. The aim of this study was to compare the adherence of Candida Albicans to heat cured resin and thermoplastic resin cured by injection modeling technique. Fourteen completely edentulous patients were selected. New complete dentures were fabricated where seven patients received conventional heat cured dentures (Group I) and Group (II), received thermoplastic dentures. Swabbing technique and germ test tube were used for C. Albicans isolation and identification following two and four months of denture insertion. Student's t-test was used to compare between the two groups. Heat cured resin showed statistically significantly higher mean colony forming unit (CFU) counts than thermoplastic resin (p<0.05). Both groups revealed a significant increase in CFU counts by time (p<0.05). Despite of the significant increase in Candida albicans CFU of heat cured resin compared to thermoplastic resin, a higher % of Candida albicans CFU to thermoplastic resin was clear (p<0.05). Hence it could be concluded that Candida albicans adherence to dentures is unavoidable, therefore, strict oral hygiene measures is required

Keywords: Candida Albicans, heat-cured resin, injection molded resin.

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INTRODUCTION

Poly methyl methacrylate resin (PMMA) is the most commonly used denture base polymer owing to its desirable physical and esthetic properties; in addition to easy processing technique [1]. Poor mechanical properties, allergic reactions to residual monomer, porosity and microbial adhesion are major problems that affect the clinical performance of prostheses [2].

Microbial adhesion onto surface porosities of dentures creates favorable conditions for microbial colonization [3]. Candida albicans is among the most important opportunistic pathogens that can colonize PMMA. The adherence of C. albicans to dentures is considered as the principle step in the initiation aggravation and recurrence of denture stomatitis [4-6].

Continuous search and efforts to improve PMMA properties has led to emergence of new processing techniques such as light-cured, microwave-cured and the injection molding techniques [7-9]. Furthermore, new polymeric materials known as thermoplastic resins were introduced. They include: thermoplastic PMMA, Nylon (polyamide) and polyacetal [10].

Thermoplastic PMMA is fully polymerized acrylate, with special blend of polymers to increase the impact strength. It is recommended in cases with repeated denture fracture owing to higher degree of flexibility than conventional acrylic resins which makes it practically unbreakable. Also, it is indicated as alternative for allergic patients due to the absence of residual monomer. The material is characterized by long-term stability, dense surface and smooth structure [11].

Clinically, denture bases are exposed to biofilm formation, accumulation of plaque and the adherence of Candida albicans [12-13]. Several studies were concerned with the adhesion and the factors affecting C. albicans adherence to different PMMA denture base [14-15]. Hence, the objective of this study was to compare the adherence of Candida Albicans to heat-cured resin and thermoplastic resin cured by injection modeling technique.

MATERIALS AND METHODS

Fourteen edentulous patients willing to construct new complete dentures were selected at National Research Center (NRC) dental clinic. Patients participated in this study regardless of their gender. The inclusion criteria were: (i) age between 55-65, (ii) healthy firm mucoperiosteum without any signs of inflammation or flabby tissues and (iii) oral salivary secretions were within average amount and consistency "thin viscid film. The exclusion criteria were: (i): systemic or neurological diseases or drugs that affect health of oral tissues and (ii) smokers were also excluded.

The participant agreed to co-operate, follow the recommendations and instructions of the clinician and they shared in the study only after a written informed consent was signed. The study protocols was reviewed and approved by the Ethics Committee at NRC. Patients were divided randomly into two groups according to denture base material used in dentures construction. (Group I) patients received conventional heat cured upper and lower dentures while (Group II) patients received thermoplastic injection modeled dentures.

Treatment protocol:

- Preliminary impressions were made using stock edentulous trays and irreversible hydrocolloid impression material. Next, border molding was performed using custom trays with modeling compound, and secondary impressions with zinc oxide and eugenol impression material was obtained. Jaw relation record was recorded using occlusion rims and wax registration method. Try-in was performed to verify the vertical dimensions of the occlusion, centric relation records and esthetics. Finally, dentures were processed, disinfected and delivered to the patients.
- Post-insertion appointments for adjustments were scheduled until the patients were free of tissue irritation. All patients were instructed to maintain denture hygiene using soft denture brush and they were recalled after 2 and 4 months respectively.

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Dentures fabricated using conventional heat-cured acrylic resins were polymerized according to the manufacturer instructions. Then, dentures were finished and polished using wet rag wheel and pumice. On the other hand, thermoplastic resin dentures were constructed using injection molding technique (table 1). Spruces and small flashes were removed carefully and the denture bases were then finished and polished using wet rag wheel and pumice.

Table (1): Denture base resins and their processing techniques

Denture resin	*Processing type*	*Polymerization Procedure*	*Powder/Liquid-Ratio*
Acrostone (WHW plastic, England packed by Anglo Egyptian Lab)	Heat activation fast heat	Pack and press curing at boiling water 100 for 20 min	21/6 ml
Bre-Crystal (Bredent- Germany)	Heat activation	Injection-molding 260 °C for 26 min. Pressure: 5 bar	Single component

- **Isolation and Identification of Candida albicans.**

Swabbing technique and germ test tube were used for C. Albicans isolation and identification after three and six months of denture insertion respectively. Swabs were obtained by vigorous rubbing of all fitting surface of maxillary dentures using sterile cotton tipped wooden swab for each patient and inoculated immediately in sterile glass tube containing Sabourauds Dextrose Broth.

20 µ from each tube of Sabourauds Dextrose Broth was placed on Sabourauds Dextrose Agar plate to which chloramphenicol was added (40 gm dextrose, 10 gm peptone, 15 gm agar and 1000 ml distilled water) and they were spread using a sterile bent glass rod. Then plated were incubated at 37°C for 48 hours ,after which all resulting yeast colonies were counted and identified by germ tube test.

In germ tube test , yeast colonies was suspended in 0.5ml of human serum , the mixture was incubated at 37°C for 2 hours , and then a drop of suspension was transferred to a glass slide and covered wit To be examined under the light microscope for germ tubes (germ tube test).

Statistical Analysis

Student's t-test was used to compare between the two groups during the follow-up period. One-way analysis of variance (ANOVA) followed by Bonferroni's test was performed to study the changes in C. albicans counts by time. The significance level was set at $P \leq 0.05$. Statistical analysis was performed using (IBM® SPSS® Statistics Version 20).

RESULTS

Quantitative analysis of data revealed absence of C. albicans prior to dentures insertion for both groups. After 2 and 4 months, heat cured resin showed statistically significantly higher mean colony forming unit (CFU) counts than thermoplastic resin group. However, both groups revealed a statistically significant increase in CFU counts by time (fig 4). As regards the % increase in CFU counts, conventional acrylic resin denture base group showed statistically significantly lower mean % increase than thermoplastic resin.

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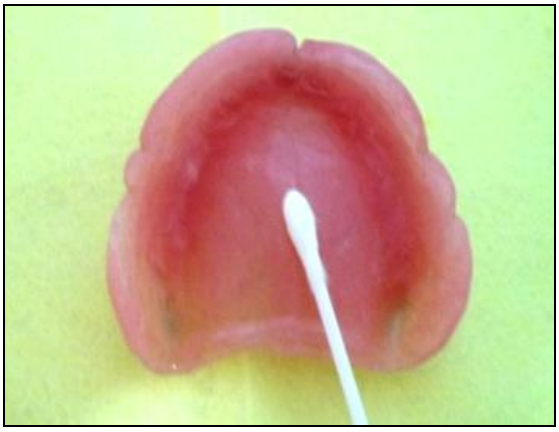

Fig (1): Denture swap

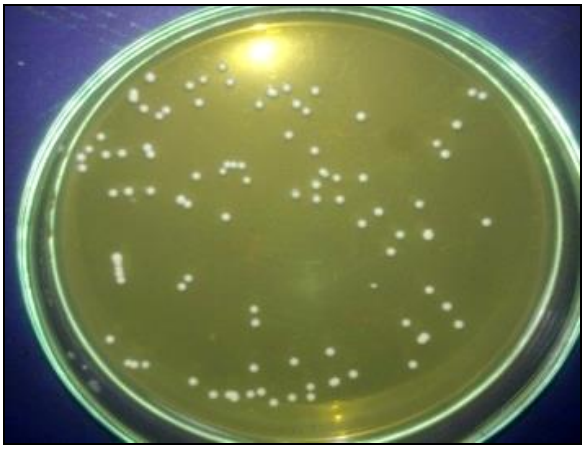

Fig (2): Positive candidal result

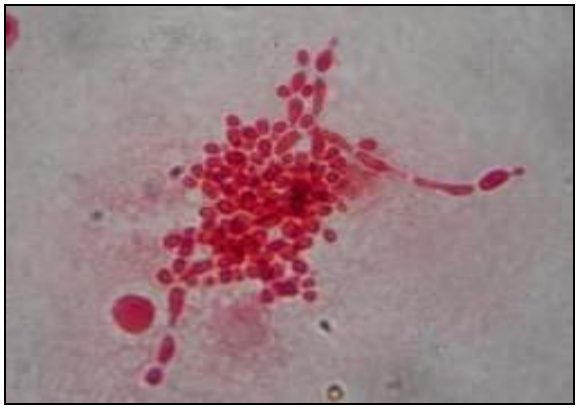

Fig (3): Germ tubes.

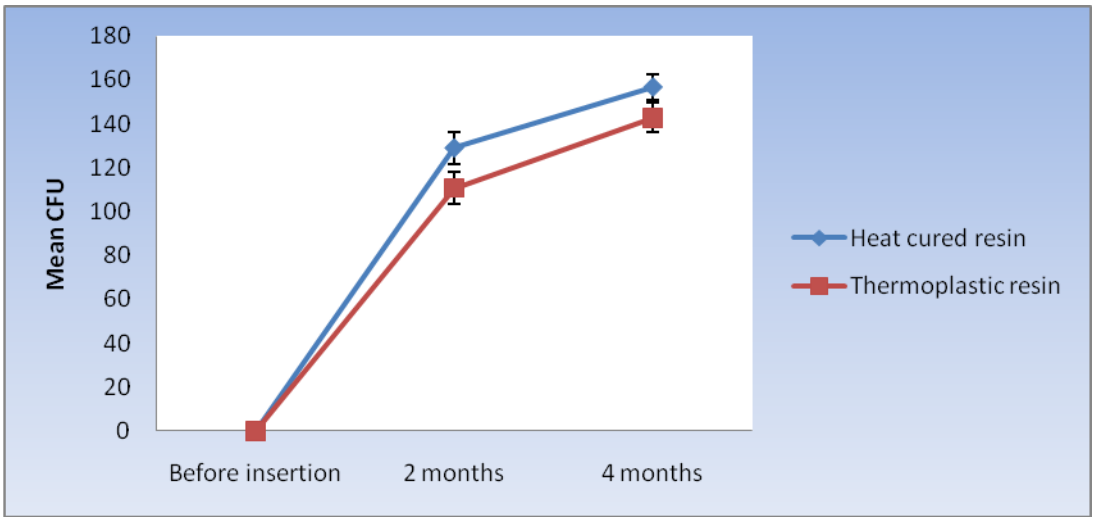

Fig (4): represent the changes in mean C. *albicans* CFU count overtime for heat cured resin and thermoplastic resin

DISCUSSION

Denture stomatitis is an oral disease associated with the presence of adherent Candida albicans. The prevalence of denture stomatitis, which has a multifactorial etiology, is high among denture wearers [16-19]; where the impression surface of a maxillary denture in particular acts as reservoir for microorganisms.

Candida albicans is the most important and predominant oral fungal pathogen. It has the ability to adhere and proliferate on both soft and hard tissues, forming a complex biofilm structures which is crucial for development of candidal infection. [20]

This study was carried out on patients wearing complete dentures to ensure clinical relevance. Two denture base materials were used; conventional heat cured resin which is used routinely for edentulous patients and newly introduced thermoplastic injection molding resin. A special emphasis on oral hygiene instructions was given to patients using soft denture brush to minimize microbial accumulation on the dentures [21].

The results of this limited study clearly show that, Candida albicans were attached to both denture base materials during the follow-up period. However, a significant increase in Candida albicans colonies to conventional heat cured resin was found compared to thermoplastic resin. This could be explained on the basis that the development of denture stomatitis is influenced by, among other factors, the denture base material [21]; in which the chemical composition of base material is important in determining the ability of pathogenic yeast cells to attach and form biofilms [22].

Furthermore, an association between oral candidosis and the duration of denture wear was reported [23]. Similarly the results of this study showed an increase in CFU count colonies count with time for both denture base materials [21].

On the other hand, a higher % of Candida albicans attachment to thermoplastic resin was clear. This may indicate other factor such as surface characteristics of the denture base material may play a role in promoting candidal adhesion [22]

Accordingly, the results of this study point out that further consideration should be given to the hygiene instructions and maintenance programs for denture wearers and should be strictly followed and performed to avoid stomatitis.

CONCLUSION

Within limitations of this study the following could be concluded:

1. Despite of denture base material used, Candida albicans were isolated from the maxillary dentures.
2. Colonization of Candida albicans on denture surfaces increased with time. Hence, strict denture hygiene procedures should be followed.

Recommendations:

Further attention should be given to researches concerned with modification and development of improved denture base materials.

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