
The Effect of Different Denture Base Materials on Microbial Flora Growth

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ABSTRACT

Objectives: The effect of different denture base resin materials on the supporting structures and oral flora was studied extensively. These materials can be used for patients with irregular or extensively resorbed ridges, as well as, ridges covered with thin, non resilient mucosal tissues. They can, also, be used in cases of bruxism, xerostomia, in dentures opposing natural teeth, patients who cannot tolerate conventional hard denture bases and for geriatric patients. Proliferation of microbial flora is affected by the surface texture and roughness of the fitting surfaces of the complete denture, hence they occur in different percentage relating to different denture base resin. **Purpose:** The present study evaluates candida albicans proliferation with complete dentures constructed with different denture base materials. **Materials and methods:** Thirty patients received complete dentures divided into three groups, Group A utilizing complete dentures constructed with heat cured acrylic resin, Group B utilizing complete dentures constructed with soft acrylic denture flanges and base) and Group C having complete dentures with casted metal base. The follow-up period was four weeks for all groups. **Results:** Significant differences were observed in the colony counts between the different base materials during the follow up period. **Conclusion:** patients utilizing complete dentures constructed with metallic denture base proved to be effective in decreasing the fungal growth typically present in complete dentures. Although overt clinical manifestations were not present, colony counts of Candida species were high in the acrylic resin denture bases of these patients. This investigation demonstrated that metal base complete dentures provide an important alternative dental service for edentulous-and other patients who are particularly prone to higher incidences of fungal infections. , moreover, more satisfaction and comfortability of patients utilizing soft acrylic resin denture base were reported.

Key words: Complete dentures, denture base materials, candida albicans.

Introduction

According to berg (Berg and Ingebretsen, 1984), construction of complete dentures depends greatly on technical, biological and physiological interactions between the prosthodontist and patients. The majority of patients were satisfied with the retention and stability of their complete dentures.

Most of completely edentulous patients is still treated by means of conventional full dentures. In spite of using dental implants as an advanced treatment plan for completely edentulous patients (Dodds *et al.*, 2005; Marsh and Martin, 1992; AlBaker, 2013; Özdemir *et al.*, 2006; Nazliel *et al.*, 2012; Darvell and Clark, 2000).

The denture bases are either metallic denture base constructed either from precious alloys (gold), non- precious metallic alloys (cobalt chromium or Nickel chromium) or non-metallic denture base that are constructed from polymers. The choice of such polymers are based on their availability, strength and durability, satisfactory thermal properties, processing accuracy, dimensional and chemical stability, color stability , insolubility and low sorption in oral fluids, absence of taste and odour, biocompatibility, natural appearance, adhesion to plastics, metals and porcelain, and easy of fabrication and repair (Anusavice, 2003).

Although PMMA based resins are not ideal in every respect, the combination of properties such as working characteristics, minimum expense, excellent esthetics, accuracy of fit, stability in the oral environment, and ease of processing account for their popularity and universal use (McCabe *et al.*, 1990).

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Human oral cavity micro-flora plays an important role in maintaining a healthy oral mucosa in normal teathed and even edentulous denture users (Hamalainen *et al.*, 2004). These microorganisms are highly diverse containing about 700 bacterial and fungal species of which more than half have not yet been isolated and differentiated (Collee *et al.*, 1996).

The importance of the condition of oral denture hygiene in regulating oral Candidal carriage have been investigated, denture plaque accumulating on the tissue surface of the denture as a result of poor denture hygiene, acts as a substrate that mediates *Candida albicans* colonization. Also. Traumatic injuries produced by an ill-fitting denture may reduce the tissue resistance against infection and increase permeability of the epithelium to soluble Candidal antigens and toxins (Budtz-Jørgensen, and Isidor, 1990)

Aim of the study

This is vivo investigation compared the oral candidal proliferation between heat-cured acrylic resin, soft acrylic denture base and metallic denture base in complete dentures.

Materials and methods

This study was carried out in the Department of Removable prosthodontics, Faculty of Oral and Dental Medicine, and microbiology department, Faculty of medicine, Cairo University. The population of this clinical study constituted of 30 patients who have been treated with complete dentures. All patients agreed with the trial protocol and signed a voluntary consent agreement.

A protocol of oral hygiene and home care of the prostheses was implemented to minimize the variables normally seen in clinical studies. The mean age of the patients ranged between 45 and 66 years with a mean age of 54 years. Patients with history of chemotherapy, radiotherapy, hormonal therapy and under any kind of medical treatments, severe ridge resorption and those with severe bony undercuts or bony exostosis were excluded. The dentures constructed were at least, six months after the last tooth extraction.

Patients were divided into three groups, Group A utilizing a hard heat cured acrylic, Group B utilizing a soft acrylic resin in base and Group C utilizing complete denture with metallic denture base.

Conventional complete denture construction process were done for all groups, starting with taking upper and lower primary impressions using irreversible hydrocolloid impression material. Self cure acrylic resin special trays were constructed then secondary impression using elastomer impression material were taken.

The dentures for Group A were processed using heat cured acrylic resins following long curing cycle, 8 hours in 160°. For Group B, technical specifications were considered to fabricate complete dentures with soft acrylic according to the manufacturer's directions for flanges and base. The acrylic resin trial denture base was kept in place to create a space for the soft acrylic resin, the first trial packing of heat cured acrylic resin was carried out and covered by a cellophane paper then the flask was closed and the excess was removed. The second trial packing was done after remove the cellophane paper and the trial denture base from the flask. A soft acrylic resin was packed in the space of the flask. Fig (1).

For Group C utilizing metallic denture base. After taking the primary and final impression. The final cast derived from this impression was duplicated and refractory cast was formed. The refractory cast is used for the fabrication of the customized metal palate. A sheet of green spacer wax was adapted on the palatal portion of the cast covering the crest of the ridge and extending 2-3 mm beyond it. Loops of 2-3 mm length made of wax were attached to the peripheral border of the previously adapted palatal spacer wax. It was taken care that all these loops were 2-3 mm short of the sulcus. These loops would enhance the interlocking of acrylic. This was important because no loop should interfere with the acrylic border of the denture extending into the sulcus. Tiny loops were placed slightly palatal to the crest. The height of these loops was such that it would not interfere with the arrangement of artificial teeth but was sufficient enough for inflow of acrylic resin. A butt joint was created palatal to the crest at the junction of acrylic and metal that enhances the strength of the metal acrylic junction creates a smooth joining of acrylic with metal avoiding any step formation, thus

making it comfortable for the patient. For making the butt joint, a 2-mm cylindrical blue wax beading was adapted palatal to the crest of the ridge. A bard parker knife was run 45° to the border of the bead. Once the design of the palate in wax was done, wax sprues were attached and casting was done. The metal palate after finishing was placed now on the master cast and occlusion rim was made for jaw relations. The jaw relations were recorded and teeth arrangement was done. After curing, the metal denture was finished and polished.

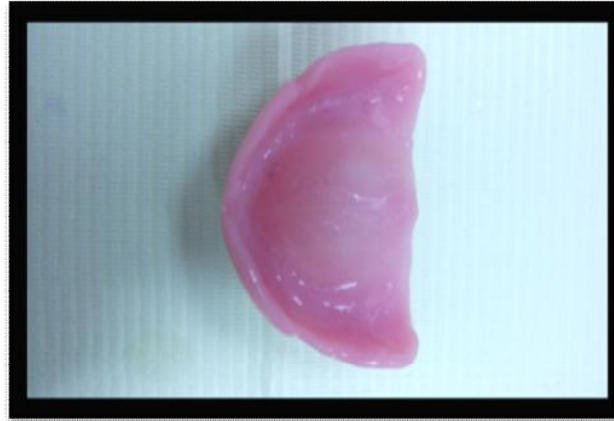


Fig. 1: Denture with soft denture base

At delivery, instructions for proper denture hygiene were stressed upon then the patients were recalled for post insertion care and further need for adjustment.

Methods of evaluation

Patient satisfaction, where Patients were asked about their comfort and denture looseness. *Clinical evaluation*, where the supporting structures of the lower ridges were clinically examined for any signs of inflammation, and *Microbiological evaluation*, the oral microbial flora changes evaluated at time of delivery and after 1 month.

The samples were collected in the morning hours between 10-12 O'clock giving chance for the patients to use the dentures several hours before taking the swabs. Patients were instructed not to eat any food before taking the swabs. The swab was taken from the fitting surface of the denture base using sterile swab. The swabs were taken from each patient in the following intervals:

- First swab, at delivery time.
- Second swab, one week later
- Third swab, two weeks later.
- Fourth swab, three weeks later
- Fifth swab, four weeks later.

In this study, three Petri dishes were used for every single swab. One petri dish for the hard cured acrylic denture swap, the other one for the soft dentures swap, and one for metallic complete denture base, Petri dishes were containing sabouraud's agar media, The swabs taken at delivery was considered as a control for each patient.

The swab was heavily seeded to give a "Well" on one quadrant of the plate by rotating the swab and sweeping over the surface several times, secondary and successive dilutions strokes were made by a sterile loop.

The wire loop was sterilized using the *Bunsen flame* so that the whole length became red-hot, then it was left to cool and was drawn from the Well into three parallel lines on to the fresh surface of the medium and this process was repeated.

Aerobic culturing:

Petri dishes for each patient containing Sabouraud's agar media, were incubated in an electrical incubator adjusted at 37°C for 48 hours.

After incubation, the plates were checked for the presence of growth and evaluated semi-quantitatively using the method of Collee *et al.* (1996) as follows:

- No growth: if no growth was detected on the line of streaking.
- Light growth: where growth occurred on the first line of streaking.
- Moderate growth: where growth occurred on the first and second lines of streaking.
- Heavy growth: where growth occurred in the first, second and third lines of streaking.

Different cultures were described for the shape and color of their colonies. The colonies were identified under the microscope according to direct smear technique previously described by Budtz-Jørgensen and Isidor, (1990) and Jawetz *et al.* (1991) using Gram stain. Fig (2).

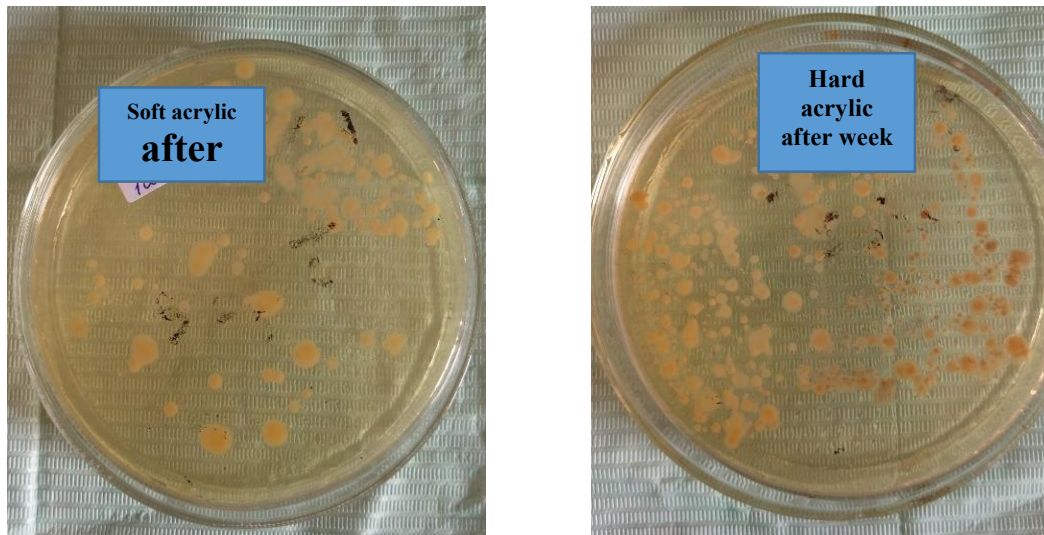


Fig. 2: Petri dishes showing the difference in growth percentage for candida albicans after one week.

Germ tube test:

A colony of the yeast was touched lightly by the loop and the yeast cells were emulsified in 0.5 ml of serum in a small test tube with loose cotton wool plug and incubated at 37°C for 2-4 hours. Formation of pseudo-germ in serum confirmed the presence of *candida albicans*.

The data was collected and statically analyzed. Statistical analysis was performed with SPSS 14.0* (Statistical Package for Scientific Studies) for Windows.

Results

The Candida Albicans count data obtained were expressed as log (CFU+1)/mL. The mean and standard deviation (SD) values of conventional denture base and soft denture base were obtained at fixed follow up periods (delivery, one, two, three and four weeks after).

The comparison between all groups was performed using one way analysis of variance (ANOVA) followed by LSD post hoc test (least significant difference) for multiple comparisons at different time intervals. One, two, three and four weeks after, there was significant increase in log candidal count of conventional denture base in all groups as P-value < 0.05 except for casted base metal group which was insignificant as P-value > 0.05, listed in table (1) and showed in figure (1).

For evaluation of effect of time on each group, One way analysis of variance (ANOVA) test was performed followed by Tukey's post hoc test for multiple comparisons which revealed that there was highly significant difference between different time intervals as P value < 0.05, listed in table (1) and showed in figure (2).

Table 1: Comparison of Log₁₀ of Mean (M), standard deviation (SD) values of Candida Albicans count for all groups:

M±SD	Candida Albicans Log (CFU+1)/mL		
	Conventional Denture Base	Soft Denture Base	Casted Base Metal Denture Base
One Week After	3.9 ^a ±0.21	2.16 ^a ±0.3	0.94 ^a ±0.82
Two Weeks After	7.33 ^b ±1.013	6.44 ^b ±0.03	1.03 ^a ±0.96
Three Weeks After	12.84 ^c ±2.34	9.51 ^c ±1.2	1.77 ^a ±0.83
Four Weeks After	19.2 ^d ±0.67	11.8 ^d ±2.3	2.01 ^a ±0.74
P-value	0.00**	0.00**	0.06*

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M; Mean, SD; Standard Deviation, P; Probability Level.

Same superscript letter in the same column indicated insignificant difference.

Different superscript letter in the same column indicated significant difference.

* Insignificant Difference

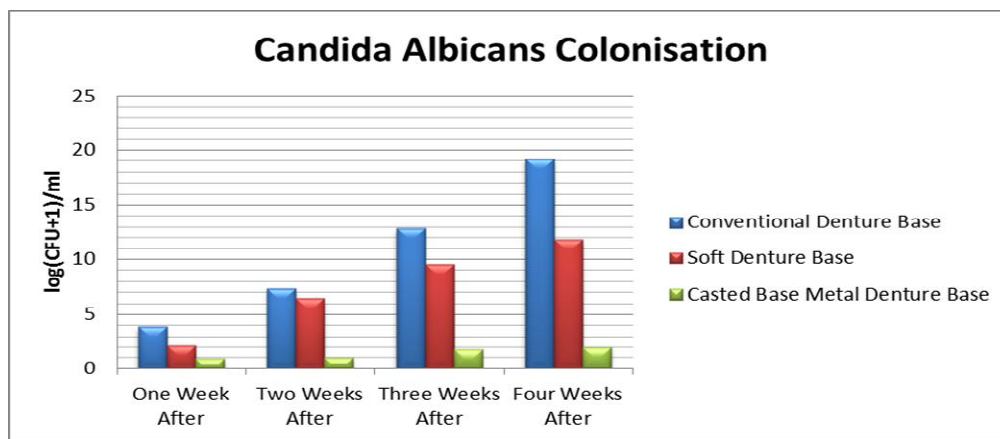


Fig. 2: Bar chart showing log count of Candida albicans for all groups

Discussion

Thirty patients were selected in this study to assess the amount of microbial colonization that can adhere to different denture fitting surfaces materials used in such patients, several criteria as sex, age, health state and others were taken into consideration. To eliminate the effect of age changes on the microbial oral flora, all patients selected ranged between 45-65 years old Jawetz *et al.* (1991).

The time elapsed between the last extraction and denture construction was not less than six months to allow sufficient time for remodeling of the underlying residual alveolar bone. All patients had no previous dentures to exclude the possible effect of the old denture on the microbial oral flora.

The alveolar ridges were well developed and cases with severe bone resorption, extreme undercuts and thin or hyperplastic mucosa were not included, as it can cause alteration of oral microbial flora (Duncan *et al.*, 1985)

The oral mucosa was free from any signs of inflammation, ulceration and swelling, which may be associated with the predominance of certain types of organism, for example, in denture sore mouth, staphylococci increases from 23% to 47% and candida from 3% to 33% (Reenen, 1973)

In spite of the accumulated biofilm on the fitting surface of the denture may be displaced by the salivary flow and movement of the denture but both the upper and the lower dentures were chosen for sampling to ensure precisely the accumulation of the bacteria on the fitting surfaces of both upper and lower dentures.

Detection of bacterial colonization on the fitting surfaces of both dentures was expected as they act as a barrier that prevent salivary cleaning by major glands and allow creating a stagnant environment on the tissue surfaces that enhances adhesion of microorganisms to be examined during the study period.

Long curing cycle was followed in processing the dentures to avoid the high levels of residual monomer, degree of porosity and stresses induced in the denture base (Lefebvre *et al.*, 1994; Burnett *et al.*, 1976).

At the time of delivery, all patients received the same instructions regarding the use of the denture as they must remove the denture at night to give chance for oral tissues to recover. Patients were also instructed to clean their dentures after each meal under tap water, with no other chemical or mechanical means that might affect plaque accumulation and microbial flora.

The samples were taken in the morning between 10-12 a.m. to fix the time of collection and before taking any kind of food to neglect the effect of meal on microbial flora.

Some investigators obtain the swab by rubbing once for a distance of 1 or 2 cm or by vigorous rubbing for an unspecified period of time. Vigorous rubbing was performed in the present study, however, to ensure a more accurate and standardized isolation procedure, the rubbing was done for a specified period of time (30 seconds).

The second, third and fourth swaps were taken on the intervals of two, three and four weeks after date of insertion, to give chance for denture adjustment and to make sure that the patients were comfortable in wearing the new denture.

Evaluation of the study data showed that the metal base surface exhibited significantly less growth of *Candida* species when compared with the heat-cured acrylic resin surface over time. This is consistent with a previous report by Pryor, (1928) who noted an inhibitory action on the growth of microorganisms when using metal bases. The clinical significance of this short-term pilot study was to use the qualities of the metal base complete denture as a treatment alternative to patients who are prone to higher incidence of fungal infections.

Also, previous results may be due to tissue surface of the denture to be considered as an irregular surface as it usually shows micro pits and micro porosities that harbor microorganisms that are difficult to remove by mechanical methods.

This study introduced soft heat cured acrylic resin as a satisfactory material to be used for complete denture wearers, who showed satisfaction, retention and comfortability to those patients that require a lining material in terms of reduced microbial colonization.

Another factor enhancing bacterial growth is the temperature increase and washing effect of the saliva below the dentures. There is direct relation between the degree of dentures retention and the increase of the temperature below the dentures. The better the retention the higher the temperature and the less the washing effect of the saliva. This situation is clear in the maxillary dentures. However, in group B utilizing soft heat cured acrylic resin dentures, the retention was better in the patient's point of view, yet, it looks that the fitting surface of the dentures is more hygienic, so that is why it overcomes the effect of the increased temperature under the dentures on the bacterial growth.

Regarding the difference in the degree of roughness and surface texture of the materials studied, this was not within the scope of this study and it may be considered in another investigation.

The results of this study also showed that there was a gradual increase in the CFU values of the total aerobic count during the time of using the dentures for these patients this gradual increase may be because the mouth dealt with them as a foreign body and reacting by increasing the CFU of total aerobic microorganisms. Although, the increase of colonization of microorganisms during the follow up period might also be due to surface roughness of the materials that should slight increase in the mean values from the beginning of wearing the dentures, as aging process promotes the surface roughness of the soft lining material.

Conclusion

Hard Heat cure polymethylmethacrylate denture base resins showed more adherence of Candidal cells compared to metal base and/or cure resins. so it can be concluded that the use of soft denture bases for completely edentulous patients would be much comfort, satisfied and retained in addition to less dentures stomatitis occurrence.

Limitations of the study

The study did not use patient's saliva which could help in simulating oral environment. Saliva plays a very pivotal role in the adherence of Candida. It consists of enzymes, proteins, antibodies, Immunoglobulins (esp. IgA) and most importantly flow which controls the growth of microorganisms in oral cavity. Other limitations include small sample size, metal denture base and other types of denture base materials which were included in the study.

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