Candida albicans colonization on different polymeric denture base materials in controlled type II diabetic patients

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Background/aim
Growth of the immunosuppressed population has provoked increased incidence of oral candidiasis infections. This study was conducted to evaluate colonization of *Candida albicans* upon the fitting surfaces of three different polymeric denture base materials in patients with controlled type II diabetes mellitus. The investigated resin denture base materials were the conventional heat-cured methacrylate and two types of commercially available flexible denture base materials.
Materials and methods
A total of 30 completely edentulous patients with controlled type II diabetes mellitus were included in the study, and they were divided into three equal groups (10 each), where group I patients received upper and lower complete dentures fabricated from conventional heat-cured acrylic resin, group II patients received a flexible versacryl upper complete denture and a lower heat-cured acrylic resin denture, and group III patients received a thermoplastic nylon upper complete denture and a heat-cured acrylic resin lower denture. Samples of *C. albicans* culture swabs were taken after 1 month and after 3 months of dentures insertion. Culture swab was obtained by scrubbing the fitting surface of all upper dentures at tuberosity area. At each time interval, three swab samples were collected from each patient and were inoculated in three individual culture media. Results of the study were statistically analyzed to evaluate the fungal colonization in each of the three investigated groups.
Results
The present results indicated that mean number of *C. albicans* colonies upon heat-cure acrylic resin denture base material at 1 month was 45±6.86, whereas at 3 months, it was 97±12.02. The mean number of fungal colonies for versacryl denture base material after 1 month was 12.80±4.80, and at 3 months, it was 22.50±6.16. The mean number of *C. albicans* colonies for nylon-based denture base at 1 month was 7.00±2.30, and at 3 months, it was 13.50±3.02.
Conclusion
Within the limitation of the study, flexible polymeric denture base materials showed lesser *Candida* adherence upon upper denture fitting surface than that of conventional heat-cured acrylic resin. In addition, nylon-based denture showed less colonization to *Candida* than versacryl denture material. Flexible dentures seem to be promising owing to their minimum microbial colonization and relatively healthier biological tissue reactions in comparison with the heat-cured acrylic resin.

Keywords:
Candida albicans, controlled type II diabetic patients, polymeric denture base materials, thermoplastic nylon

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Introduction

In recent decade, growth of the immunosuppressed population has provoked increased incidence of oral *Candidiasis* infections. Oral moniliasis (i.e. oral candidiasis) is a common opportunistic fungal infection of the oral mucosa because of overproliferation of *Candida* species and in particular the *Candida albicans* [1,2]. Moreover, diabetic patients are considered more susceptible than others to oral *Candidiasis* when certain predisposing factors are present, such as sex, age, nutritional disorder, poor oral hygiene, dentures, smoking habit, salivary pH alteration, and xerostomia (i.e. mouth dryness) [3,4].

It has been estimated that ~85–90% of diabetic patients were diagnosed with type II diabetes mellitus (DM, i.e. caused by insulin resistance). Diabetic patients had an increased predisposition to oral diseases including *Candidiasis*, which was frequent with uncontrolled hyperglycemia associated by therapeutic resin dentures [5–7].

Presence of removable prostheses in patient mouth might decrease the salivary flow rate, might alter the saliva pH, and might impede mechanical cleansing of the oral soft tissue surfaces with the tongue movements. Moreover, the fitting surface of acrylic resin denture offered niches that harbored oral microorganisms and enhanced their pathogenic infective potential [8].

In 1931, Dr. Wright introduced the heat-cured conventional polymethyl methacrylate (PMMA) acrylic resins to construct removable dentures. PMMA properties had contributed to success of that denture base material, including cost effectiveness, availability, easy handling and processing, proper fit, minimum biodegradation and good stability in oral environment, and reparability [9]. Despite the properties of heat-cured PMMA materials were acceptable, they were not absolutely ideal in all dental aspects, like unyielding severe undercuts, very pronounced tuberosity, torus palatinus and/or mandibularis, and bulging alveolar bone ridge. Moreover; PMMA had some inherent limitations, like low fracture toughness values, impact strength, flexural strength, fatigue strength, hardness, and reported allergic tissue reactions [9]. Therefore, variable approaches had been attempted to improve the physicomechanical properties of conventional heat-cured PMMA denture base materials [9,10].

Flexible dentures were recently introduced in the dental market aiming to minimize localized pressure areas, to achieve even masticatory force distribution, and to improve denture base retention by intimate adaptation to the supporting oral tissues and engagement of hard and soft tissue available undercuts [11,12]. In addition, versacryl as a modification of cross-linked resin denture base material is available as self-cured and heat-cured polymers, and no special curing equipment is needed for its processing. Indeed, nylon being a generic name of certain types of thermoplastic polymers, it belongs to the polyamides class. Compared with PMMA denture base materials, nylon polyamides were flexible, strong, highly resilient, less rigid, light weighted, comfortable, virtually invisible, less allergic, durable, unbreakable and resistant to surface abrasion [13,14]. Despite the continuing development of the flexible denture base materials, *C. albicans* adherence of these improved materials has not been yet investigated [15].

This study was conducted to evaluate colonization of *C. albicans* upon the fitting surfaces of three different polymeric denture base materials in patients with controlled type II DM. The investigated resin denture base materials were the conventional heat-cured methacrylate and two types of commercially available flexible denture base materials.

Material and methods

Materials

Alginate impression material was purchased from Cavex CA37 (Cavex Holland BV, Holland, Netherlands).
Plaster of Paris was purchased from Moldano. Bayer, W. (Germany).
Auto-cure acrylic resin was purchased from Acrostone (England).
Rubber base elastomeric impression materials were purchased from Coltene, Whaledent, Speedex (Switzerland).
Pink wax was purchased from Cavex Holland Bv, Haarlem, Noord-Holland, (Netherlands).
Modified anatomic acrylic resin artificial teeth were purchased from Acrostone Dental & Medical Supplies (Cairo, Egypt).
Heat-cure acrylic resin was obtained from Acrostone Dental & Medical Supplies.
Flexible acrylic resin was obtained from 'Versacryl' (Keystone Industries GmbH, Sigen, Germany), nylon (bre.flex2, http://www.eschoenitz.co.uk/wp-content/uploads/2019/02/thermopress-400_000626GB-20180119.pdf), and CHROM agar Candida medium from CHROM agar, France.

The instruments utilized were face bow (Whip Mix #8645 Quick Mount, Louisville, Kentucky, USA), semiadjustable articulator (Hannau, Modd H, Teledyne, Buffalo, New York, USA), and injection molding machine thermopress (Fig. 1).

Patients

Thirty completely edentulous patients were selected from the Prosthodontic Department in the Excellence Centre, National Research Centre (NRC). All patients had controlled type II DM, were males, had age ranging from 50 to 60 years old, were nonsmokers, and were not receiving any medication other than the oral hypoglycemic treatment. Medical records for all patients included in the study were taken concerning the fasting serum glucose level, which was higher than 126 mg/dl, as well as the glycosylated hemoglobin level, which does not exceed 7.5%. Patient examination was done, including extraoral and intraoral examination, panoramic radiograph, and diagnostic casts. Moreover; the selected patients were skeletally Angle's class I, and

Figure 1

Injection molding machine for thermopress polymer.

they had upper and lower well-developed alveolar ridges covered with firm mucoperiostia. Patients were instructed to get dentures out during the night and clean, to massage the oral tissues and denture fitting surface with tooth brush daily at night, and to keep denture in a cup of water over the night.

The 30 study patients received upper and lower complete dentures that were fabricated from conventional heat-cured acrylic resin denture base material. Maxillary and mandibular preliminary alginate impressions were made for every patient, then, they were poured to obtain the diagnostic casts upon which; special trays were constructed with auto-cure acrylic resin. Afterward, accurate border molding and final impressions were made using rubber base elastomeric impression materials that were poured to get the master casts.

Moreover, the occlusion biting blocks were constructed on the prepared upper and lower final casts. Aided by a maxillary face bow, the maxillary master cast was mounted on a semiadjustable articulator, whereas the mandibular final cast was mounted by the centric jaw relation using the wax wafer technique. Modified anatomic acrylic resin denture teeth were set up with the application of the concept of lingual occlusion. Furthermore, try-in of the constructed waxed up dentures was done in the patient's mouth. The patients were classified into three equal groups (10 patients each) as follows:

Group I

Maxillary and mandibular complete dentures were processed into heat-cured acrylic resin using the long curing cycle (curing at 70°C for 9 h).

Group II

The maxillary complete dentures were fabricated from flexible acrylic resin 'versacryl,' which was packed in flasks and processed similarly to method of the heat-cure acrylic resin. A conventional heat-cured acrylic resin mandibular denture was processed.

Group III

Every patient received a maxillary thermoplastic nylon upper complete denture and a conventional heat-cured acrylic resin mandibular denture. Evidently, the injection molding technique was implemented for processing of the thermopress upper nylon complete denture (Fig. 1) Initially the 'Start-Heating' key was pressed to start liquefying the nylon polymer crystal cartridge to 222°C for 15 min. After complete softening of the nylon crystals, the 'Start-Injection' key was activated aiming to press the thermoplastic polymer material throughout the sprue channel into the denture mold of the flask.

Whenever the injection technique was completed, the system had stopped for minutes, and then the machine piston was moving backward, and the flask was removed from the injection molding machine by pushing the 'Eject' key, therefore deflasking of denture, followed by finishing and polishing at low speed using thermal resin finishing burs and pumice. Finally, buffing was done to give a very high luster to the denture, and it was ready for insertion.

Ethical considerations

At beginning of the study, patient's data (personal, medical, and dental) were collected according to the implemented principles of research ethical committee of NRC, and written consents were obtained from the

participating patients. All patients were informed about practical steps of this study and signed approval consent. This study was approved by the Ethics Committee of National Research Centre (Approval No. 16/086).

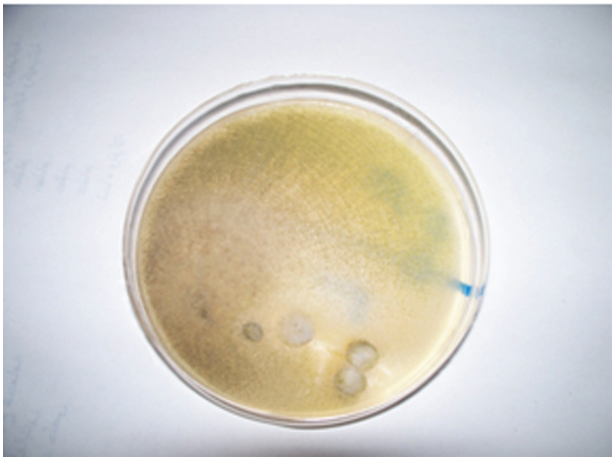
Oral mucosa swabs and isolation of *Candida albicans*

From all the study groups, morning mucosal swab samples were collected after 1 month and after 3 months of denture insertion from fasting diabetic patients. The samples were obtained by swabbing the oral mucosae at tuberosity areas that were intimately in contact with the maxillary dentures. Afterwards, mucosal swab samples were cultured on Sabouraud glucose agar plates. Initially, all isolated yeasts were counted and were identified by subculturing it on CHROM agar *Candida*, followed by performing the germ tube test and getting hyphae/pseudo-hyphae and chlamydospores growth [15,16].

Countable dilution of yeasts was visually achieved by aid of a permanent marker and by naked eyes (Fig. 2). Moreover, the number of candida colonies appearing on the Petri dish was counted (i.e. colonies forming units per sample; CFU/sample). Then, that number was converted into CFU/ml. Finally, the total number of *C. albicans* colonies per swab sample was calculated using the following equation [15,16]:.

$$\begin{aligned}\text{CFU/ml} = {} & \text{Total number of candida colonies} \\ & \text{counted in culture plate} \\ & \times \text{Inversion of saline dilution} \\ & \times \text{Inversion of the whole cultured volume} \\ & \times 1000\end{aligned}$$

Figure 2



Candida albicans colonization on Sabouraud glucose agar plates.

Statistical analysis

Results of the study were statistically analyzed to evaluate the fungal colonization in each of the three investigated groups. Statistical analysis was performed with SPSS 20 (IBM SPSS software, Watson, USAhttps://www.ibm.com/sa-en/about?lnk=fab_saen), Graph Pad Prism, and Microsoft Excel 2016, with significant level set at *P* value less than or equal to 0.05. The obtained data were presented as mean and SD values in that in-vitro comparative study.

Results

The present results indicated that in group I (heat-cured acrylic resin), mean±SD of *C. albicans* count was 44.9 ±2.3 and 97.3±1.6 after 1 month and after 3 months of maxillary denture insertion, respectively. Moreover, in group II (versacryl resin), mean±SD was 12.8±1.5 and 22.4±1.3 after 1 month and after 3 months of upper denture insertion, respectively. Furthermore, in group III (nylon, polyamide-based resin), mean±SD was 8.2 ±0.7 and 12.9±1.7 after 1 month and after 3 months of upper denture insertion, respectively (Table 1).

In addition, paired *t* test was again used to compare the *Candida* colonization after 1 month and that after 3 months, correspondingly in each study group. The implemented statistical test had revealed significant difference between the two follow-up periods in all three study groups (I, II, and III), as *P* value was less than 0.05 (Table 1, Fig. 3).

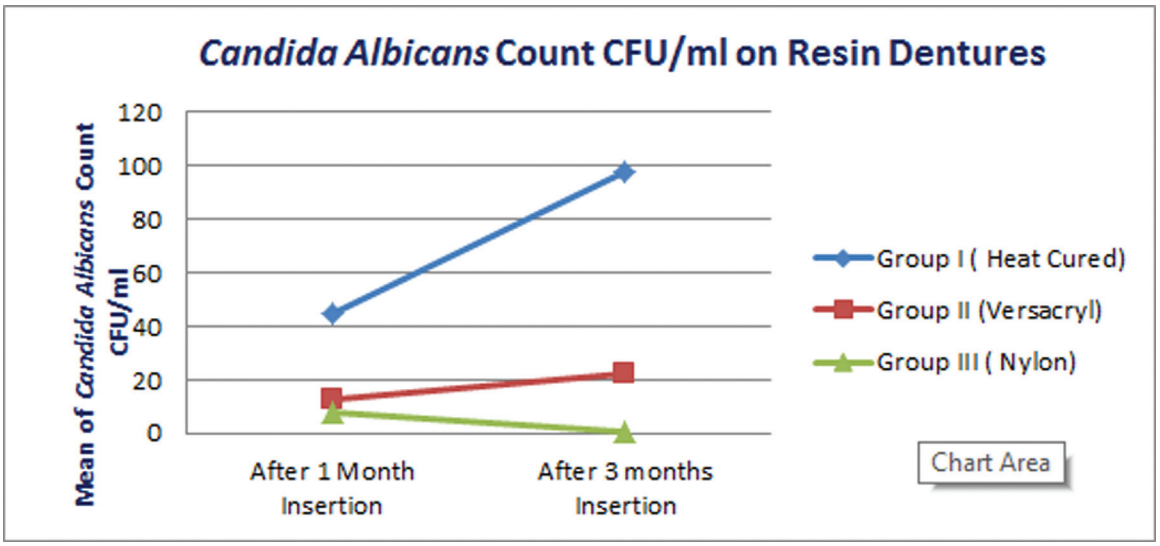
Comparison among the three groups (I, II, and III) at two follow-up periods (after 1 month and after 3 months) was performed using one-way analysis of variance test, which revealed a significant difference between all studied groups after 1 month and after 3 months, as *P* value was less than 0.05. Therefore, Tukey's post-hoc test was followed for multiple

Table 1 Mean and SD of *Candida albicans* count using three different polymeric maxillary denture base materials at two time intervals in patients with controlled type II diabetes mellitus

	Candida albicans count after 1 month of upper denture insertion (mean±SD)	After 3 months of upper denture insertion (mean±SD)
Group I (heat cured)	44.9±2.3[a]	97.3±1.6*[a]
Group II (versacryl)	12.8±1.5[b]	22.4±1.3*[b]
Group III (nylon)	8.2±0.7[b]	12.9±1.7*[c]

[a,b,c]Different letter within the same column are significant using one-way analysis of variance at *P* value less than 0.05. *Significantly difference than after 1 month of upper denture insertion group, using *t* test at *P* value less than 0.05.

Figure 3



Bar chart for three groups of variable polymeric maxillary denture base materials at two different time intervals in patients with controlled type II DM.

comparisons between the three groups after 1 month and after 3 months, which revealed a significant difference between groups I and II as well as between groups I and III, whereas it revealed insignificant difference between groups II and III regarding the *C. albicans* colonization (Table 1).

Discussion

Patients with controlled type II DM were selected in the study owing to the high prevalence of DM, as ~140 million individuals had been reported [9]. Furthermore, only the controlled diabetic patients were included in the study, aiming to guard their blood glucose level as close as possible to the normal one, because uncontrolled DM might reduce salivary flow and pH as well as increase patient salivary glucose level. Those critical factors were found to enhance growth of oral candida and its fast colonization [17].

All diabetic patients were free from any debilitating systemic diseases, such as AIDS, HBV, HCV, and anemia, which might increase the microbial tissue adhesion [18]. Moreover, smokers and patients taking any medication other than the oral hypoglycemic tablets had been totally excluded from the study, because smoking might produce a clinically undetectable mild alternation in oral mucosa, and thereby, it might facilitate pathogenesis of oral candidiasis in diabetic patients [19].

Actually, swab sampling from fitting surface of maxillary denture was preferred in designing of the study rather than that of mandibular denture. Such preference was because the accumulated biofilm (interest of swab sample) beneath the fitting surface of the lower denture might be washed out with salivary flow or it might be displaced by movement of mandibular denture [19].

Saliva of diabetic patients was favoring growth of yeast colonies, and their resin denture fitting surfaces had shown significant higher counts of *C. albicans* in comparison with nondiabetic individuals [16]. In addition, mechanical trauma to oral mucosa from an ill-fitting denture was reported to increase the probability of soft tissue penetration and colonization by surrounding *Candida* yeasts [20].

Indeed, acrylic resins had been the most widely used worldwide denture base materials. However, dental resins were economic and easily manipulated; they had shown certain limitations, such as hydrostatic forces, surface-free energy, inherent hydrophilicity or hydrophobicity of the material, as well as water sorption. Those acrylic features were affecting the nature of *C. albicans* adherence to the fitting surface of denture base materials and were even altering the degree of adherence of yeasts to resins [21].

Adherence of *C. albicans* was correlated to the inherent resin microporosity of the denture fitting surfaces. The morphology of such polymeric denture irregularities was not only making the elimination of bacterial commensal difficult, it was also enhancing flourishing oral yeasts and fungi [22]. Those findings

were interpreting and in coincidence with the obtained significant difference in candida colonization values after 1 month and after 3 months in the three studied groups (group I: conventional heat-cured acrylic resin, group II: versacryl flexible resin, and group III: nylon thermopress resin).

According to statistical results of the study, there was a significant reduction in *C. albicans* count values for flexible denture base materials in groups II and III (versacryl and nylon, respectively) than the conventional heat-cured acrylic. Such illustrated difference in the degree of *C. albicans* colonization was most probably attributed to the variation in physical properties of the denture base materials, which might be determining the pattern of *C. albicans* adherence and consequently the density of yeast colonization [23,24].

Flexible dental resin materials were providing homogenous stress distribution, stress breaking action, and tissue conditioning in case of long-term usage. Such flexible resin advantages might be owing to their slight sliding as a result of their inherent flexibility that might allow sufficient normal blood circulation underneath the denture, and therefore, they might reduce the oral soft tissue atrophies. On the contrary, surface roughness of acrylic resin denture base was representing a critical factor that was assisting in entrapment of various microorganism in the oral cavity [25–27].Microbial colonization to fitting surface of conventional heat-cured acrylic was significantly higher than its colonization to flexible denture base materials. Such finding was attributed to presence of ionic surface charges upon flexible resin materials, which might be facilitating adsorption of some salivary immunodefensing molecules like histamines [24,28,29]. Such interpretation might be applicable to explain the obtained significant difference in *C. albicans* colonization values between the conventional heat-cured acrylic resin and other both flexible denture base materials (versacryl and nylon).

Statistically, there was no significant difference between versacryl and polyamide-based nylon (i.e. bre.flex2). Such finding might be attributed to high biocompatibility of those flexible resin materials, as versacryl contained few or even no residual monomer. Moreover, the biological considerations of polyamide-based nylon might be owing to the injection molding technique (thermopress, i.e. under temperature and pressure) of the processing rather than the chemical polymerization reactions [30,31].

Conclusion

Within the limitations of this in vivo comparative study, it can be concluded that *C. albicans* colonization is much more reduced on surfaces of flexible resin. The thermopress polyamide-based resin (i.e. nylon) seems to have least *C. albicans* adherence of all studied polymeric denture base material. Consequently, flexible polymeric denture base materials seem to be promising substitutes for conventional heat-cured acrylic dentures, especially for diabetic patients and for individuals who are allergic to residual monomers of conventional resin.

More *in vitro*, *ex vivo*, *in vivo*, and clinical studies are recommended to investigate the nature and different properties of the various commercially available polymeric dental materials.

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Conflicts of interest

There are no conflicts of interest.

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