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COMPARATIVE STUDY OF CANDIDA ALBICANS ADHERENCE TO CONVENTIONAL ACRYLIC DENTURE BASE MATERIALS AND INJECTION MOLDING ACRYLIC MATERIALS AND POLY ETHER ETHER KETONE

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ABSTRACT

Aim: The purpose of this study was to compare the candida adherence to injection molding acrylic resin, the poly ether ether ketone (PEEK) material and to the conventional acrylic resin.

Materials and methods: The study was conducted in-vitro and in-vivo including 3 groups, conventional acrylic resin group, injection molding acrylic resin and the PEEK groups. The in-vitro study included 60 discs (10×10×2 mm), 20 discs for each group, *Candida albicans* ATCC-10231was cultured and *Candida* adherence was evaluated by colony count method. The in-vivo study included 30 participants with maxillary partial dentures, 10 participants for each group, wearing their dentures for at least 3 months. The swabs were obtained from denture fitting surfaces and cultured to investigate *Candida* adherence by colony count method.

Results: Both in-vitro and in-vivo results showed the highest *Candida* adherence in case of conventional acrylic resin material (330±35×10^8 cfu) in-vitro and (287.53 ±68.53 square root / cfu) in-vivo. It was significantly decreased in case of injection molding resin (226±15 ×10^8 cfu) in-vitro and (221.89 ±44.31 square root/cfu) in-vivo, while PEEK showed no *Candida* adherence in-vitro and in-vivo.

Conclusions: PEEK material has been shown to be bio-inert, and when combined with injection moulding resin, it provides a more biocompatible alternative to acrylic resin.

KEYWORDS: *Candida*, biofilm, denture base materials, biocompatibility, PEEK

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INTRODUCTION

Candida albicans is an oral commensal that colonizes the posterior part of dorsum surface of the tongue, palate and other soft tissues and it can also adhere and colonize the hard dental surfaces. [1] Under certain conditions, including systemic alteration of immune system or local oral environment together with *Candida* virulence factors, *Candida albicans* becomes pathogenic and may induce *Candida* infection with variable clinical presentations [2].

Denture stomatitis is a common clinical presentation of the *Candida* infection that can be manifested in denture wearers, whether they have complete or partial dentures [3]. The *Candida* adherence to denture base is the essential step of biofilm formation, and it is influenced by physical properties and surface characteristics of denture base material [4] such as roughness [5], porosity, surface free energy and hydrophobicity [6]. These are all related to the type of denture base material and technique of fabrication in terms of polymerization method, surface modifications and incorporation of surface coating or fibers [7].

Biocompatibility is a critical feature that should be provided by the denture base material as the oral cavity is rich in microorganisms that tend to be adsorbed on polymers which can provide them with carbon and oxygen. This adsorption may result in the production of biofilms, which can lead to diseases such as candidiasis, caries, and periodontal inflammation [8].

Conventional acrylic resin material is considered the material of choice for denture base construction, especially in developing countries, due to their ease of construction and low cost regardless of their shortcomings due to poor mechanical properties, allergy related to excess monomer [9] and susceptibility to microbial colonization, biofilm formation and denture stomatitis induction [10].

Over the last two decades, injection moulding acrylics have become one of the most popular denture base materials because the technique of their fabrication provides many advantages over the conventional compression moulding, including dimensional stability, proper tissue adaptation, and is less laborious and error-prone [11]. The elasticity, flexibility and absence of residue of these materials make them more biocompatible with less plaque adhesion [12].

Poly Ether Ether Ketone (PEEK) is a semi crystalline high temperature thermoplastic biomaterial that shows excellent thermal and mechanical properties. It has many biocompatible proprieties such as being non-toxic, non-allergic, low plaque affinity [13], and have good polishing properties. All these properties make PEEK suitable for variable medical and dental applications [14, 15] .

Many research studies have compared injection molding acryl versus conventional acrylic resin [16-19]. Only one study has investigated *Candida* adherence to PEEK versus flexible [20]. No study has investigated the *Candida* adherence of PEEK versus conventional acrylic resin to our knowledge. In order to provide more biocompatible alternatives, this study compares *Candida* adherence to injection moulding acrylic denture base materials and PEEK materials versus conventional acrylic resin in-vitro and in-vivo.

MATERIALS AND METHODS

Study design and ethical statement

It was a two-part clinical comparative study *in-vitro* and *in-vivo*. The Faculty of Dentistry Beni-Suef University Research Ethics Committee approved the protocol with approval number (#FDBSUREC/11062020/ER). For the *in-vivo* study, all the participants were informed about the study details and signed their written consent.

In-vitro study

The *in-vitro* part was carried out in a private Dental lab in Giza, Egypt. It included sixty discs that were divided into 20 conventional pressure

pack acrylic discs (group 1), 20 injection molding acrylic discs (group 2) and 20 PEEK discs (group 3). All the discs were constructed according to manufacture instructions with standardized dimensions (10×10×2 mm) (Figure 1) [18]. The disks were polished with a particular sequence of bures and stones, by a technician with controlled hand pressure in order to reach nearly the same polished surface in all disks

Candida count

All *in-vitro* and *in-vivo* experiments to assess *Candida* adherence were carried out in a private microbiological laboratory in Beni-Suef, Egypt. *Candida albicans* ATCC-10231 (Oxoid, UK) was used as a test organism. ATCC -10231 was cultured on sabouraud dextrose agar (SDA, Oxoid, UK) plates that were incubated at 37°C for 48 hours. Then fungal suspension was prepared from SDA plates and standardized to 1×10^8 cfu/ml in a sterile normal saline to achieve the equivalent turbidity of 0.5 McFarland standard.

A 100 µL of this suspension was placed on the surface of each disc of the three groups. Each sample was incubated at 37˚C for 1 hour and then washed with normal saline for 5 seconds to remove non-adherent cells. Attached cells were removed from the discs by shaking at 3000 rmp for 20 seconds. Discs then were stained with crystal violet stain and the *candida albicans* was quantified by using the colony count method **(Fig. 1)**.

A total of 10 random fields were viewed under the light microscope by 100 power lens for each sample. After 10 random fields, number of cells were counted and were tabulated to be used for statistical analysis.

In-vivo study

From July 2020 to May 2021, 30 participants were chosen from the outpatient clinic of the Removable Prosthodontic Department at Beni-Suef University, Faculty of Dentistry and a private dental clinic in Giza, with 15 patients from each clinic. Participants were divided into three groups, each with ten patients: conventional acrylic denture wearers (group 1), injection mould denture users (group 2), and PEEK denture wearers (group 3).

All participants were partially edentulous patients with free end saddles (Class I Kennedy) having the first or the second premolars as the last standing abutments with maxillary partial dentures. They should have worn their dentures for at least three months, had no significant medical history, were nonsmokers, had not used broad-spectrum antibiotics or corticosteroids in the previous three months, and had good oral hygiene (Plaque index ≤ 1) [21]. They were also told to brush their remaining teeth three times a day and to follow the denture hygiene recommendations, which included brushing

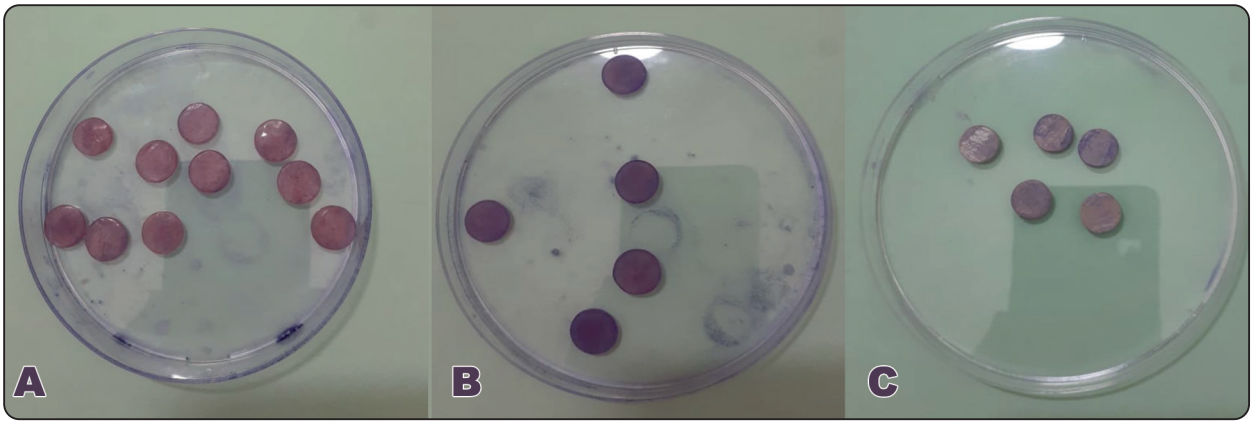

Fig. (1): Comparison of Stained discs

their dentures with a soft brush and soaking them overnight in an antibacterial denture cleaner (Corega tablets denture cleaning) [22].

The sample collection was done in the morning by gamma sterilized swab applicator (HP5007, Snapple PS stick material and cotton head, PP tube material, Jiangsu Huida Medical Instruments). The samples were obtained by vigorous scrapping of the denture fitting surface for 30 seconds to ensure collection of denture plaque and the swabs were immediately kept in their sterile tubes and transferred to the lab.

The swabs were cultured on sabouraud dextrose agar (SDA) plates and incubated at 37°C for 48 hours, after which *Candida albicans* was detected using Gram stain as well as germ tube test and examined under light microscope for identification where yeast cells were dark purple and displayed characteristic budding. Colonies were counted and tabulated for statistical analysis.

Statistical analysis:

The statistical package for the social sciences (SPSS) version 26 was used to code and enter the data (IBM Corp., Armonk). The mean and standard deviation were used to summarize the data. For non-normally distributed data, a square root transformation was used. The analysis of variance (ANOVA) with multiple comparisons post hoc test was used to compare the groups [21]. P-values less than 0.05 were considered as statistically significant.

RESULTS

In-vitro Study

On comparison, the conventional acrylic resin discs revealed the highest Candida adherence (330±35×108 cfu) and the injection molding acrylic discs revealed a significant decreased Candida adherence (226±15 ×108 cfu, p value <0.001), while the PEEK discs revealed no Candida adherence (0±0, p value <0.001) **Fig. 2** and **Fig. 3**.

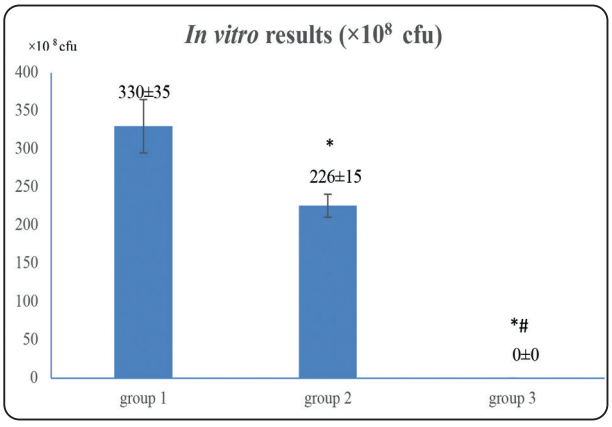

Fig. (2): Comparison of in vitro Candidal adherence to conventional acrylic resin discs (group 1), injection molding resin discs (group 2) and PEEK discs (group 3)

: Statistically significant compared to corresponding value in group I (P<0.05)

#: Statistically significant compared to corresponding value in group II (P<0.05)

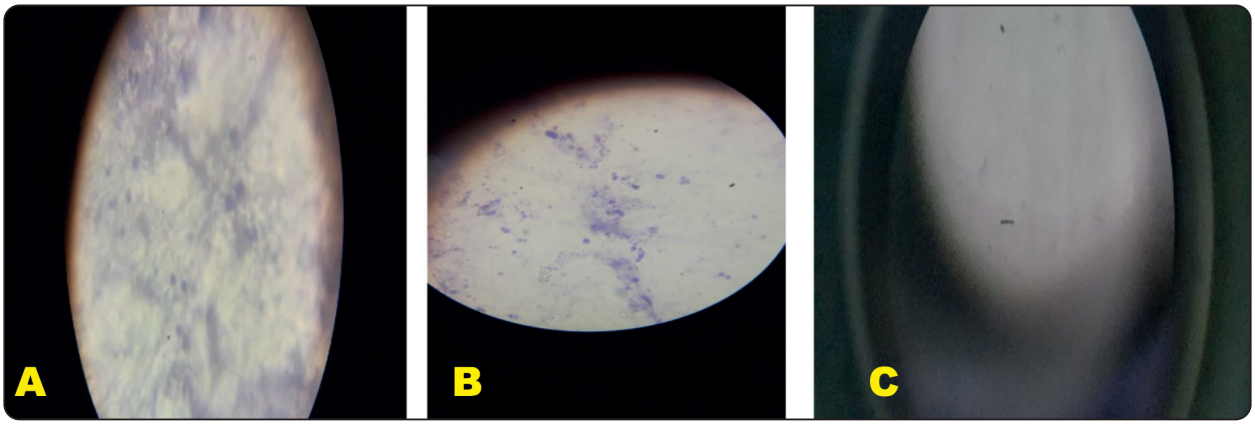

Fig. (3): Comparison of in vitro Candidal adherence under light microscope, (a) Conventional acrylic discs, (b) Injection molding discs and (c) PEEK discs

In-vivo Experiment

The *in-vivo* results are shown in **Fig. 4.** The comparison is the same as *in-vitro* as the conventional acrylic resin dentures revealed the highest candida adherence (287.53 ±68.53 square root/ cfu) followed by injection molding resin with significantly decreased *Candida* adherence (221.89 ±44.31 square root /cfu, *p value < 0.001*), while PEEK dentures revealed no *Candida* adherence.

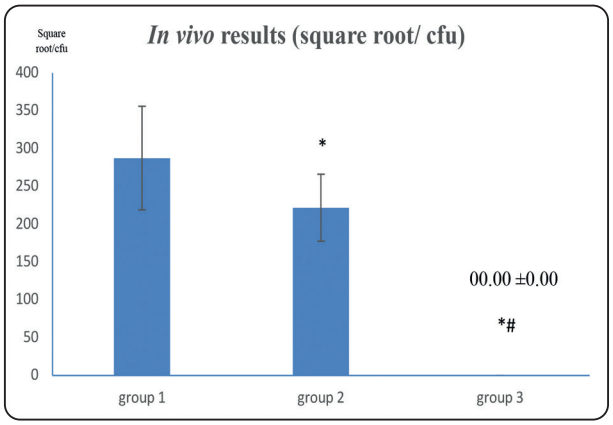

Fig. (4): Comparison of in vivo Candidal adherence to conventional acrylic resin discs (group 1), injection molding resin discs (group 2) and PEEK discs (group 3)

Values are presented as mean ±SD

****: statistically significant compared to corresponding value in group I (P<0.05)***

#: statistically significant compared to corresponding value in group II (P<0.05)

DISCUSSION

The adherence of *Candida* to the denture base is the essential step of biofilm formation and subsequent exposure of oral mucosa to microbial toxins which is responsible for variable degrees of denture stomatitis [22]. It can be directly or via denture plaque and it depends mainly on surface characteristics and composition of the denture base, microbial physicochemical characteristic and the regulatory role of saliva that might be attributed to physical washing effect and its anti-candidal components of innate immunity [23].

The current study investigated the Candida albicans species adherence, in both in vitro and in vivo parts, as it is the most frequent species to be isolated from biofilm associated with denture stomatitis and has a varying degree of adherence to acrylic resin denture base materials [24]. The in vivo study was carried out to consider the role of saliva and other possible factors that can modulate the Candida adherence to denture base as time of contact [23].

Denture wearers are at a higher risk of oral *Candida* overgrowth beneath the dentures due to decreased salivary flow, potential mucosal alteration from mechanical and chemical irritation, and the denture base acting as a reservoir for microorganisms and a substrate for microbial biofilm. Since, the incidence of biofilm development is relatively high in maxillary partial denture wearers than in mandibular partial denture wearers [25, 26], the current study included maxillary partial denture wearers for at least 3 months to provide enough time of contact and excluded those who had any possible factors that could increase the *Candida* count, such as smoking, broad-spectrum antibiotics and corticosteroids intake, systemic conditions, and poor oral hygiene [27].

The conventional acrylic dentures (heat cured polymethyl methacrylate (PMMA) polymers) are the most commonly used types of denture base material due to their cost effectiveness and ease of construction with acceptable mechanical and physical properties [28]. However, the inherent micro porosity of resin and surface roughness provide shelter for Candida adherence that might be accelerated by surface hydrophobicity and electrostatic interactions to develop the microbial biofilm [29]. This explains the highest Candida adherence of the conventional acrylic resin in the current study, (330±35×108 cfu) in-vitro and (287.53 ±68.53 square root/ cfu) *in-vivo*.

The approaches to overcome the inherent

problems of conventional acrylic resin dentures are either to improve the composition of PMMA, reinforce with other materials or provide alternative materials [30] as done in the current study. The injection molding acrylic material revealed significantly lower *Candida* adherence than conventional acrylic resin, (226±15×108 cfu, p value <0.001) in vitro and (221.89 ±44.31 square root/ cfu, p value <0.001) in-vivo. These results are in consistence with other studies *in vitro* [17,18], *in vivo* [20,31]. These results might be explained by improved physical properties of the injection moulding acryl due to modification of polyamide and injection molding technique using cartridge reducing the contraction with subsequent stability of shape and increases mechanical resistance and homogenous stress distribution [32]. *In-vivo* outcomes could be explained further by inherent flexibility, which allows normal circulation beneath and less trauma to soft tissues, as well as increased charges on flexible surfaces, which adsorb salivary immune molecules such as histatin, which has anti-Candida effects [33].

In the current study, PEEK materials revealed no *Candida*'s adhesion whether in vitro (0±0) or in vivo (0±0) and significantly lower than both conventional acrylic resin and injection molding acryl (p value <0.001). To the best of our knowledge, there have been no studies comparing *Candida*'s adherence to traditional resin and PEEK; nevertheless, a study comparing Candida's adherence in PEEK versus flexible dentures found *Candida*'s adhesion in both materials, but substantially lower in PEEK [34]. These findings can be explained by high biocompatibility properties of PEEK, which include being non-toxic, non-allergic, enhancing cellular proliferation and tissue healing, and being hydrophobic, which prevents protein adhesion and subsequently has a low plaque affinity [35]. Further investigations are recommended for *Candida*'s adherence to PEEK material both *in-vivo* and *in-vitro*.

CONCLUSIONS

From the results of this study, it can be concluded that, Poly ether ether ketone (PEEK) is indeed a bio-inert material with low plaque affinity that, when combined with injection molding acryl, can be considered an excellent alternative to conventional acrylic resin dentures regarding *Candida's* adhesion, particularly in denture wearers who have a high risk of candidiasis due to uncontrolled diabetes or poor oral hygiene.

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