

**Original Article**

# Evaluation of Candida Albicans Growth on Bre-Flex Versus PEEK Denture Base in Bilateral Maxillary Bounded Partial Denture: A Randomized Clinical Trial

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## Abstract

**Aim:** This study aimed to compare the adhesion of Candida Albicans to the fitting surface of Bre-Flex Versus PEEK removable partial denture bases and mucosa beneath them, , in patients with maxillary class III modified 1 Kennedy's classification

**Subjects and methods:** Eighteen patients having Kennedy class III modification I Maxillary partially edentulous ridges with fully dentate mandibular arch were divided into two equal groups to fabricate the thermoplastic materials. The first group received Bre.Flex material. The second group received PEEK material. For each studied group, microbiological evaluation was carried-out according to the follow-up recall visits: at the denture insertion, three weeks and four weeks after denture insertion. The collected data were tabulated and statistically analysed.

**Results:** showed that the difference between the two groups was statistically significant was reported between two groups regarding microbiological evaluation.

**Conclusion:** Candida Albicans can grow on both denture base material (Bre-Flex and PEEK). Swabs collected from oral mucosa in Bre-Flex group shows higher number of Candida colonies in comparison to the number of colonies in PEEK group

**Keywords:** PEEK, thermoplastic materials, microbiological evaluation , Candida.

## Introduction

A successful removable partial denture must meet important basic principles of retention, stability, support, and affordable cost, beside to restoring functions and aesthetics according to the patient's desires. Most removable partial dentures are used for the replacement of missing natural teeth are

fabricated from metallic and/or acrylic components.<sup>1</sup>

Esthetics in the anterior region can be obtained by using a removable partial denture over a fixed restoration, especially when there is loss of soft/hard tissues surrounding the abutment teeth. If a <sup>metal</sup> clasp arm of the denture terminates in the undercut of a tooth in an aesthetic zone area, this will result in

poor esthetic. Metal-free removable partial dentures made of thermoplastic materials are biocompatible, nonirritant, nontoxic, comfortable, biologically inert, and with superior esthetics. Several types of non-metal clasp dentures are available, all with the advantages of superior esthetics and the reduced potential for allergic reactions to metals. Additional advantages of these dentures are their flexibility and highly elastic nature, which decrease the stress on abutment teeth.<sup>2</sup>

In 1936 the first thermo-polymerisable acrylic resin was developed. Acrylic resins are synthetically obtained materials, the polymerization of which is through chemical reaction. They are known as poly (methyl methacrylate) or PMMA. During their initial phase, acrylic resins can be modeled, packed or injected into molds.<sup>3</sup>

Bre.flex is a nylon-based thermoplastic material. Chemically, the original nylon is a PA 12 (polyamide). Bre.flex exhibits excellent flow characteristics due to the low melting temperature. The thermoplastic material is processed under a pressure of 7.0 bis. The high pressure reduces shrinkage and ensures extended dimensional stability so that precision-fit dentures are obtained and accumulation of plaque is avoided.<sup>4</sup>

Polyaryletherketones (PAEKs) are a group of high-performance semi crystalline thermoplastic resins, which family members differ according to their ratio of keto- and ether-groups .With a higher ratio and sequence of keto groups, the rigidity of the polymer chain and the glass, as well as melting temperature are increasing.<sup>5</sup>

The denture clasps made of PEEK have lower retentive forces compared to cobalt-chromium (Co-Cr) clasps. However, since the study was conducted on metal crowns in vitro, it is not known how effective the esthetic PEEK clasps would be in retaining dentures in the clinical setting.<sup>6</sup>

Peek dental implants is used nowadays instead of titanium dental implants because of hypersensitivity to titanium, difference in the modulus of elasticity of a titanium implant and its surrounding bone that may cause stress in the implant-bone interface during load transfer, resulting in peri-implant bone loss.

Also, titanium can cause esthetic problems in cases of thin biotype mucosa.<sup>7</sup>

PEEK is used in various dental applications mainly are: dental implants, implant abutments, fixed crowns, fixed bridges and removable dentures.<sup>8</sup>

Oral fungal infections are frequent, especially *Candida albicans* which is a commensally in the oral cavity of 45-65% of healthy individuals. That is, due to its high virulence, ability to adhere and form biofilms on oral cavity tissues. In denture wearers, the prevalence of *Candida* increases to 60-100%<sup>9</sup>

*Candida albicans* is a dimorphic fungus since it grows both as yeast and filamentous cells.

It is a common member of human gut flora and does not seem to proliferate outside mammalian hosts.<sup>10</sup> It is detectable in the gastrointestinal tract and mouth in 40-60% of healthy adults., It is usually a commensal organism, but can become pathogenic in immunocompromised individuals under a variety of conditions.<sup>11</sup>

Mechanical oral cleaning such as tooth, tongue or denture cleaning reduces the number of oral microbes and risk of oral infection or aspiration pneumonia<sup>12</sup>

Two major approaches are generally recommended to patients for the removal of plaque from their dentures. Dentures can be cleaned mechanically or chemically. Mechanical methods include brushing (with water, soap, toothpaste or abrasives) and ultrasonic treatment. Chemical methods are classified according to their composition and mechanism of action (e.g. hypochlorites, peroxides, enzymes, acids and mouth washes (oral rinses) for dentures)<sup>13</sup>

This study aimed to compare the adhesion of *Candida Albicans* to the fitting surface of Bre-Flex Versus PEEK removable partial denture bases and mucosa beneath them, in patients with maxillary class III modified 1 Kennedy classification .

## Subjects and Methods

Eighteen patients were selected from the outpatient clinic of Prosthodontic department, Faculty of dentistry, Cairo University. All patients were have Kennedy class III modification I Maxillary partially edentulous ridges with fully dentate mandibular arch.

The study was designed to be a parallel randomized controlled trial. In terms of internal validity, randomized clinical trials represent the most scientifically rough study design, when properly performed, as they are best able to control bias and serve as a gold standard of study designs for evaluating treatment efficacy and widely considered as highest level of confirmatory scientific evidence.

The patients were randomly assigned to either one of two groups by using a special web site concerned with randomization process called research randomizer

[\(https://www.randomizer.org/\).](https://www.randomizer.org/)

Only one investigator, not involved in the selection and treatment of the patients, was aware of the randomization sequence and could have access to the randomization lists stored in his password protected portable computer. The randomized codes were enclosed in sequentially numbered, identical, opaque, sealed envelopes. Patients were asked to select one of envelopes and the investigator that is aware about the randomization process was asked about the specific group and treated accordingly; the first group: Patients received maxillary removable partial denture fabricated from Injectable Bre.Flex second edition (Bredent, 2<sup>nd</sup>, Germany) resin material reinforced by metal framework. While the second group: Patients received maxillary removable partial denture fabricated from injectable polyetheretherketone (PEEK) (GMbH&Co.KG.Germany) reinforced by metal framework.

A maxillary partially edentulous ridge (Kennedy class III with modification 1) with good tooth anatomy was used because maxillary arch is more esthetic than mandibular arch. Premolar area was chosen because it is important for the prosthesis to be retentive and clasp placed on the canine is more favorable to be constructed from esthetic material as it may appear during patient smiling.

Preliminary impressions were made for the patient's maxillary and mandibular arches by using irreversible hydrocolloid (alginate) (Cavex CA37 Alginate impression material, Holland BV). Impressions were disinfected, then were poured in improved dental stone (Elite® rock dental stone, Zermack, Italy). Primary surveying was done on the diagnostic casts, and then face-bow record was used to mount the maxillary cast on semi adjustable articulator (A7 plus, Bio-Art Dental

Products, São Carlos, SP, Brazil.). Diagnostic casts were mounted on a semi adjustable articulator to check for any teeth interferences. This was important to evaluate interarch distance to accommodate the future prosthesis. The mounted casts also were used to assess the antero-posterior jaw relation.

Panoramic and periapical radiographs were performed as a complete mouth survey to evaluated the bone index areas and crown root ratio. The selected patients were informed about the participation in scheduled follow-up for 1 month after receiving the removable partial denture by a written informed consent. An informed consent was signed by each patient as it is one of the most important facets of bioethics to make sure that a patient understands the risks and benefits of any medical procedure. (Ilfeld 2006) stated that - Requiring informed consent protects many patients from being forced to participate in medical studies without understanding the risks involved Mouth preparation was made including guide lines preparation and rest seat preparation.

The maxillary final impression was taken using medium-bodied elastomeric impression material (AquasilMonophase, DENTSPLY CAULK, USA) mixed according to the manufacturer's instructions. Impression was boxed and poured, in extra hard dental stone (Elite® rock dental stone, Zermack, Italy). The master cast was modified by drawing the design which is palatal strap major connector, Aker's clasps were drawn on the molars and Aker's arm was drawn on the canine then duplicated into a refractory cast by using silicon (Technosil, Bredent, Germany) to fabricate the metal framework .The use of Removable partial denture made from combination of thermoplastic resin and metal is now rapidly gaining popularity among general dentists and is considered to be superior to conventional metal-clasp retained RPDs with metal clasps in terms of both esthetics and comfort as the rigidity of the metallic framework distribute the forces equally and thermoplastic clasps enhance the esthetic so these type of RPD made a combination of esthetic and mechanical point of view.

The PEEK materials were injected under 400°C for 20 minutes by pressure 950 mega Pascal and velocity 6 bars in 240 seconds while the Bre-Flex materials needs 222 °C for 15 minutes for injection in the injection molding unit

(Thermoflex 400). Each denture was finished and polished properly and inserted in the patient's mouth and carefully verified to ensure proper vertical dimension of occlusion and harmonious occlusal relationship.

### **Isolation of Microorganisms:**

Isolation of the microorganisms using gamma sterilized disposable cotton swabs was collected at faculty of Dentistry Cairo University after dentures' insertion.

Swabs were collected from the tissue – bearing surface of the partial denture

A rotating motion with the swab held firmly and laterally against the denture was used to collect the plaque to ensure proper adherence of the plaque.

These swabs were immediately transferred to the commercially available sterile swab holders and placed in the refrigerator for no more than two hours before plating. The previous three steps were repeated for each patient at 3 times interval: Before insertion of partial denture; (Base line) ,After three weeks and 4 weeks of insertion

#### **Culturing and incubation procedure:**

Each swab was incubated immediately in sterile tube containing 1 ml of sterile saline for fifteen minutes, and so undiluted samples were achieved.

Using the pre-adjustable micropipette, a 20  $\mu\text{L}$  of the undiluted sample was then plated on the SDA medium. Then the sample was spread using a sterile glass rods to facilitate the platting and spreading procedure.

The plates were covered and left for one minute to dry and then were inverted immediately before being placed in incubator for 24 hours at 37 °C.

Culture plates which were negative for candida growth were left for further 48 hours and then examined again before being discarded as negative sample.

#### **Identification and counting of candidal colonies:**

Number of *Candida* colonies was determined by counting colony forming units per sample on SDA plate (CFU/sample). (Figure 1)

Primary identification of candida was carried out after growth of characteristic creamy convex yeast colonies on SDA.

If the number of colonies above 200 colony, The plates were split into sectors of equal size (lines were drawn on the bottom of the plate), all

colonies were counted in one sector and then multiplied by the number of sectors

CFU/ml is then calculated as following:

CFU/ml = Total number of colonies counted in the plate X 50

### **Results**

Statistical analysis was performed with SPSS 20<sup>®1</sup>, Graph Pad Prism<sup>®2</sup> and Microsoft Excel 2016<sup>3</sup>. All the colonies were counted in one sector and then multiplied by the number of sectors. CFU/ml = total number of colonies counted in the plate X 50. All data were explored for normality by using Shapiro Wilk Normality test which revealed that data follow normal distribution ,accordingly Independent t-test was used for comparisons. The significant level was set at P ≤ 0.05.

As shown in table 1 and figure 2 at the time of insertion the swabs taken from the mucosa and the denture respectively and the candida count from the mucosa in bre-flex group was 408 and from the PEEK group was 545 CFU/ml.

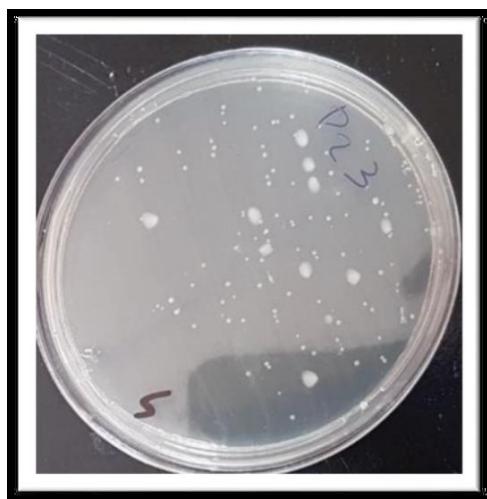
The Candidal count at third week decreased surprisingly on both the groups , from the mucosa by 49% as the count dropped from 408 to 170 CFU/ml in Bre-flex group and by 43 % as count dropped from 545 to 308 CFU/ml in PEEK group but on the other hand the candida growth increased in the samples collected from the denture surface in both groups ( in PEEK group increased from 4.15 to 83.3 CFU/ml and in Bre-Flex group from 0 to 158.3 CFU/ml.

At the fourth week the Candidal count start to increase in both groups and from mucosa and denture surface as it increased in mucosa samples in Bre-flex group from 170.8 to 775 CFU/ml ( 455 % ) and in PEEK group increased from 308.3 to 508 CFU/ml ( 164 % ), also samples from denture surface area showed increase in candida count as it increased from 83.3 to 337.45 CFU/ml ( 405.1 % ) in Bre-Flex group and from 158.3 to 541.6 CFU/ml (342.3 % ) in PEEK group

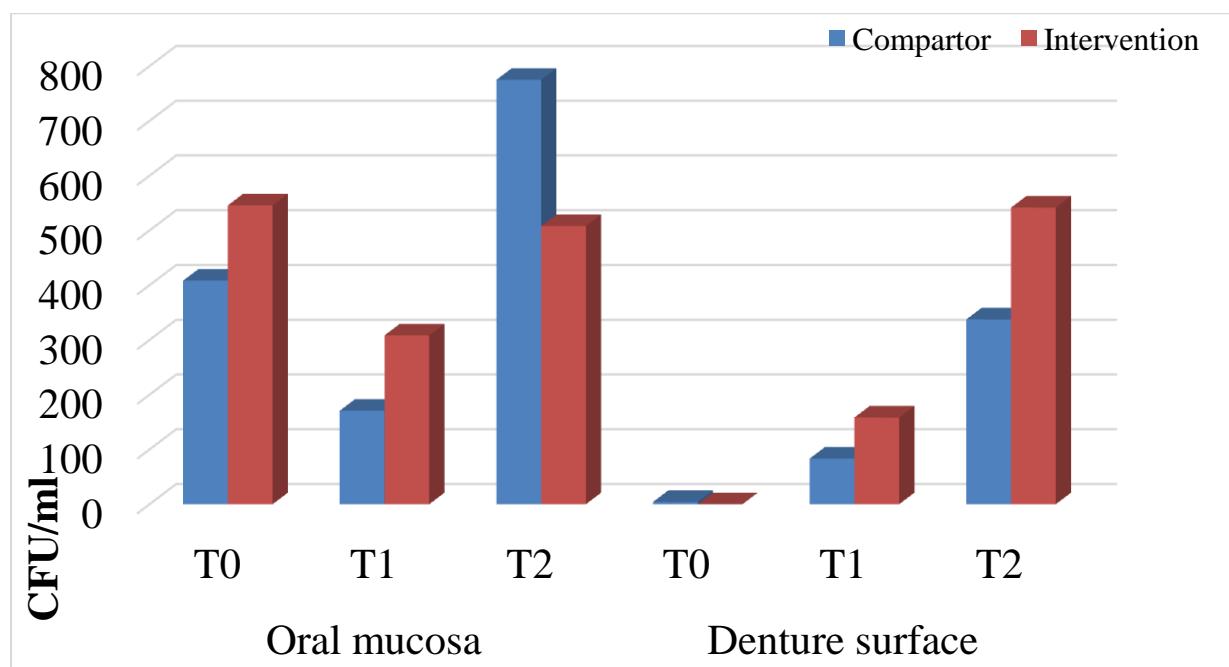
<sup>1</sup> Statistical Package for Social Science, IBM, USA.

<sup>2</sup> Graph Pad Technologies, USA.

<sup>3</sup> Microsoft Co-operation, USA.

**Figure 1 :** Petri plates with candida colonies**Table ( 1):** Comparison between swabs from two groups during follow up time

	Follow up	Comparator		Intervention		P value
		M	SD	M	SD	
Oral mucosa	T0	408.3	4.9	545.8	6.7	< 0.05*
	T1	170.8	1.09	308.3	2.6	< 0.05*
	T2	775	3.9	508	4.6	< 0.05*
Denture surface	T0	4.15	0.25	0.00	0.00	< 0.05*
	T1	83.3	0.56	158.3	0.61	< 0.05*
	T2	337.45	3.82	541.65	5.63	< 0.05*

**Figure (2):** Comparison between swabs from two groups during follow up time

## Discussion

At the time of insertion the swabs taken from the mucosa and the denture respectively, The candida count from the mucosa in Bre-flex group showed a significant difference while swabs taken from the denture shows no significant difference which changed later , the results agreed with **Periera-cenici et all 2008** that found the microbial cells have been shown capability to adhere and colonize on both oral mucosa and denture base<sup>14</sup>

The Candidal count from mucosa at third week decreased surprisingly on both groups , from the mucosa by 49% in Bre-Flex group and Peek group by 43 % as the supposed explanation of this decrease is that participants showed great oral hygiene care using mouthwashes which agrees with **Murdula Patel,et all 2008** results that shows mouthwashes containing fluoride effective in decreasing candida count<sup>15</sup> but on the other hand the candida growth increased in the samples collected from the denture surface in both groups with significant difference between the two groups which shows the effect of aging of the prosthesis on microbial colonization and change<sup>16</sup> , regarding the difference between the groups the surface characteristics like surface roughness , surface energy and hydrophobicity played great role in increasing of the count and the difference between the groups.<sup>17,18</sup>

At the fourth week the Candidal count keeps increase in both groups (Bre-flex and PEEK) also in swabs taken from both mucosa and denture surface which suggest that by aging of the denture more candida colonies can adhere to the denture and mucosa increasing the count also the neglecting of the oral hygiene increase the tendency of candida growth.

## Conclusion :

- 1) Candida Albicans can grow on both denture base material (Bre-Flex and PEEK).
- 2) Swabs collected from oral mucosa in Bre-Flex group shows higher number of Candida colonies in comparison to the number of colonies in PEEK group .
- 3) Swabs collected from denture surface in PEEK group shows higher number of Candida

colonies in comparison to the number of colonies in Bre-flex group.

4) PEEK is better regarding the candida growth on mucosa under the denture while the Bre-Flex is better regarding the candida growth on denture surface

## Conflict of Interest:

The authors declare no conflict of interest.

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