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Comparison of *Candida Albicans* Adhesion to Various Denture Base Materials

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Thesis submitted by

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Abstract

Objective: Denture stomatitis is one of the most common types of fungal infection, affecting up to 76% of complete and partial denture patients. *Candida albicans* is considered the most common fungus infection in the oral cavity and the major microorganism associated with denture stomatitis. This research examines the adhesion of *C. albicans* to the surface of seven different denture base materials and its relation to surface roughness.

Materials and Methods: Seven different types of the denture base material (Avadent CAD/CAM, Eclipse, SR Ivocap High Impact, Clear Ivocap, Lucitone FRS flexible, Nature-CRYL Pour acrylic resin, DC acrylic S.P.) were selected. Fifteen samples of each material were prepared with a standard dimension of 15 x 5 mm and a thickness of 2 mm. Surface roughness was measured using a Mitutoyo profilometer. All samples were incubated with *C. albicans* suspension and candidas adherence was counted by a plating technique. The Kruskal-Wallis test was used to detect statistically significant differences in adhesion and surface roughness. Spearman correlation analysis indicated a statistically significant association between Candida adhesion and surface roughness.

Results: The study found significant differences in *C. albicans* adhesion to the denture base material samples ($p<0.001$). Significant differences in the surface roughness of the materials were found ($p<0.001$). Spearman correlation analysis indicated that the surface roughness of the denture base material affected *C. albicans* adherence in a statistically significant way.

Conclusion: A less porous denture base material has a significantly lower *C. albicans* count.

Introduction

I. General introduction

Edentulism is defined as "the state of being without any natural teeth."[1] It is an international problem, particularly in the 65-year-old and older age groups.[2, 3] This condition is usually considered to be the result of dental caries and periodontal disease. Economic wealth, education, the availability and use of professional and preventive services, oral health care systems, third-party payment, dental awareness and social beliefs are among the factors that have been reported to influence edentulism in a given area, making it a multifactorial entity.

Oral diseases are a major health problem around the world because they may result in pain, reduced function and negative impact on quality of life. The potential increase in dietary consumption of sugars and lower exposure to fluoride has increased the risk of dental caries.[1] Oral-dental trauma also results in tooth/teeth loss, particularly in developing countries.[4] Lower education level and low income are the most important factors in the lack of access to dental health care, which also leads to tooth loss.[5]

Oral rehabilitation or restoration requires attention and expense. The treatment cost of oral disease is very high, and it is considered as the fourth most expensive medical treatment.[6] The restoration of a missing tooth/teeth can be done in many ways by using prosthetic devices.

The complete edentulous patient is defined as a patient with a complete loss of natural teeth. In this condition a complete denture is constructed. The complete denture is a dental prosthesis that restores all of the natural teeth and associated parts. Although there is a wide disparity among countries, edentulism is not limited to the developing world. Finland (46%), Kyrgyzstan (46%), Ireland (48.3%), Malaysia (56.6%), the Netherlands (65.4%), and Iceland (71.5%) report some of the highest levels of edentulism.[5]

Edentulism is unlikely to be eradicated. Limited data on edentulism rates suggest a 0.7% decline per year in Finland between 1974 and 1978, and 1% per year in Sweden between 1975 and 1996.[5] In the United States, the rate of decline over the years has been less rapid. According to Oral Health–Healthy People 2010: Objectives for Improving Health, [7] 26% of the U.S. population between the ages of 65 years old and 74 years old are completely edentulous. In 1998, Thompson and Kreisel[8] predicted about a 50% decrease in the edentulous condition for the 75-plus age group from 1990 to 2025 in the United States and Canada. A 2002 projection by Douglass[9] suggested a steady increase in the demand in the United States for treatment of edentulous arches over the next 20 years despite the declining trend in edentulism. According to this prediction, the number of edentulous arches in the U.S. is expected to increase by 230,000 units each year.

The data collected during diagnosis and examination of a complete edentulous patient should be thoroughly studied and analyzed. Additionally, the treatment plan should be made considering convenience in terms of the patient's oral condition, skill of the operator, economic status of the patient and patient cooperation. Oral rehabilitation of edentulous patients by using removable dentures needs special attention by a dentist. [9] The treatment goals of restoring the edentulous patient with a removable denture include improving esthetics, speech, and masticatory function and protection of residual structures. These goals can be achieved with the replacement of missing teeth in a completely edentulous arch that can be accomplished with:

a. Fixed prostheses (partial-complete)
b. Removable prostheses (partial-complete)
c. A combination of fixed and removable dental prostheses (attachment prostheses)
d. Support prostheses (parodontal-gingival-parodontal-gingival supported-implant).

A fixed prosthesis is designed to be retained in the patient's mouth continuously and is only removed by the dentist. A removable denture or overdenture can be removed and replaced in the mouth by the patient. There are many advantages to removable complete dentures, including:

a. Less expensive,
b. Light in weight,
c. Easily constructed,
d. Easily repaired and relined,
e. Easily adjusted.

There are also some disadvantages to removable complete dentures such as low strength, less patient tolerance and lack of retention. The removable implant assisted overdenture has become a predictable treatment modality to gain desired treatment outcomes. Implant overdentures have greater retention and improved patient comfort. The bone quality and the anatomical structure at the site of dental implants play an important role in selection of overdenture treatment. The cost of treatment of an implant overdenture may also be a limitation.

II. Removable Complete Dentures:

a. Denture base materials

While metal denture bases exist, most denture base materials are made of polymethylmethacrylate (PMMA) due to its various desirable properties,[10, 11] including being lightweight, excellent esthetics, assisted, repair and rebase, low cost and biocompatibility with oral tissues and low solubility.

Conversely, the disadvantages of PMMA include mechanical drawbacks such as low wear resistance and low flexural strength. An inappropriate percent of monomer in the mix may result in cytotoxicity for some patients due to the residual monomer.

There have been many attempts to improve the mechanical properties of PMMA through reinforcement with fibers or changes in the chemical composition of the PMMA.[12, 13] With an evolution in the field of CAD/CAM, digital dentures have also become a promising treatment. Digital dentures simplify the method for providing dentures because their application requires

fewer clinical appointments compared to traditional denture fabrication techniques. These CAD/CAM systems use either additive or subtractive manufacturing techniques for denture bases, reducing manufacturing time and cost, and are said to improve the fit of the complete dentures (Figure 1). Manufacturers claim that the denture base material is much less porous than conventional denture base materials. Along with lower porosity, this might result in easier cleaning and less harmful bio-film on the denture surface.

b. Denture care

Complete denture patients should follow a set of denture care guidelines, including daily cleaning and brushing of the dentures with a non-abrasive solution to reduce the number of microorganisms.[14] If a bio-film forms on the fitted surface of the denture, infections and/or inflammation will likely follow. Therefore, dentures are considered to be a local co-factor for infections. A further understanding of the microbiology related to this area may help in decreasing the risk of infection or occurrence of other complications.

The American College of Prosthodontics (ACP) suggests guidelines for denture care based on research evidence. They recommend the daily removal of bio-film from the oral cavity and the denture. The dentures should be brushed daily and soaked in a non-abrasive cleanser solution. This cleanser solution should be used only outside the mouth. The dentures should be rinsed with water after cleaning with the cleanser solution. There is also a recommendation to have the

denture cleaned by a dentist annually using an ultrasonic cleanser to decrease the accumulation of bio-film over time.

All patients should be instructed to avoid denture cleaning in boiling water. Cleaning with a bleaching solution such as sodium hypochlorite should also be avoided. The denture should be stored in water if not worn for an extended period of time. Denture adhesive improves the function of the dentures. Attention should be taken when using adhesives containing zinc, because this may result in an adverse effect. The denture adhesive should be used equally on all parts of the denture to maintain unique retention. The denture adhesive should be removed daily from the oral cavity and prosthesis. The denture should not be continually worn over 24 hours. An annual check of the denture is very important to insure that denture remains in an optimal condition.

c. Problems associated with dentures

While PMMA is the most commonly used denture base material, there are significant differences between the PMMA materials on the market. Certain PMMAs require specific processing techniques and the final product often has polymerization shrinkage and porosity which may affect the fit, mechanical strength and cleansability. The impaired fit and retention of the denture may lead to trauma to the denture bearing area and may increase the rate of residual ridge

resorption. This may result in compromised stability and may require frequent denture relines. Fungal infections are common among denture users.

d. Denture stomatitis

Denture induced stomatitis is an inflammation of soft tissues (gingiva/palate) under a complete/partial denture or orthodontic appliance. Denture stomatitis may be caused by fungal infection or mechanical irritation. Mechanical irritation plays a major role in increasing the proliferation of epithelial cells[15]. Denture stomatitis is reported to be the most common fungal infection, affecting up to 76% of complete and partial denture patients (Figure 2)[16, 17].

Candida albicans is considered the most common fungus infection in the oral cavity, and the major microorganism associated with denture stomatitis.[18, 19] There are some strains of *C. albicans* found with denture stomatitis, especially the *hylph* form. Denture stomatitis usually affects female more than males. Denture stomatitis usually affects elderly persons who wear removable dentures. The upper jaw is more susceptible than the lower jaw because of saliva wash and the size of the denture-bearing area. A denture stomatitis patient may complain of bleeding, swelling, a burning sensation, halitosis, xerostomia and altered taste sensation. In 1962, Newton suggested a classification for the staging of denture stomatitis: [20]

- Newton Type I: pin-point hyperemic lesions (localized simple inflammation).
- Newton Type II: diffuse erythema confined to the mucosa contacting the denture (generalized simple inflammation).
- Newton Type III: granular surface (inflammatory papillary hyperplasia).

Denture stomatitis may also be associated with some lesions of fungal origin, such as angular cheilitis, median rhomboid glossitis and candidal leukoplakia. There are many etiologic factors for denture stomatitis where it does not exist without prosthesis. The type of material used for the construction of a denture plays an important role in the development of denture stomatitis. Dentures can also change over time due to ecological changes and this may facilitate the adhesion of bacteria and yeast. There are a wide number of risk factors that may be associated with denture stomatitis, these include [21]:

1. Wearing complete dentures, maxillary more than mandibular
2. Poor denture hygiene
3. Wearing dentures for a lengthy period, especially at night
4. Poor denture quality
5. Immunodeficiency, such as diabetes mellitus, vitamin A deficiency, iron deficiency
6. Impaired salivary gland function and use of xerogenic medication
7. Tobacco and alcohol use.

The treatment of denture stomatitis is a multidimensional process and does not consist of only antifungal treatment. [22]There is no association between fungal presence in the tissue and the clinical symptoms of denture stomatitis. The treatment of denture stomatitis should focus on denture fabrication or sanitization rather than antifungal treatment in healthy denture wearers [23].

The adhesion between *C. albicans* and the host is very complex. This interaction has been suggested to be between a Candida legend and host cell receptor. These receptors are monoreceptors that contain mamillone ell protein ic36, fibrogen, fibronictine and laminin that bind with *Candida* as a result of CR3 recognition. The extracellular polymeric material contains mannoprotine that are produced by *C. albicans*. Macroscopically, Candida species are soft and

creamy similar to a yeast odor colonization. It usually grows in a PH range of 2.5 - 7.5 and temperature range of 20 - 38 °C.

The importance of yeast is in its ability to grow rapidly at 37 °C, which makes it the most pathogenic of species. Microscopically, Candida shows some dimorphism and transit from ovoid budding blastosoers to parallel side hip. Also, most of the yeast is similar under microscope and are gram positive with some changes in the form of blastosoers varying from ovoid to elongated or spherical [24].

e. Denture surface properties

The microporosity on the denture surface is a suitable environment for microorganisms. This may increase in the presence of denture stomatitis in the underlying tissues. Surface properties have a direct relation with the accumulation of plaque and adherence of *C. albicans.*[25] A study by Waters et al. found no conclusive relation between the surface energy of denture soft lining materials and the degree of *C. albicans* adherence. Microorganisms with high SFE are expected to adhere to surfaces with high SFE, whereas low SFE microorganisms adhere to low SFE surfaces.

Denture material properties, such as hydrophilicity and surface chemical properties may have a significant influence on the amount of fungal adhesion. The denture affinity for water, either absorbing or dissolving in water, is known as hydrophilicity.

Surface roughness of the denture base can be affected by the acrylic monomer/ polymer ratio and polymerization method.

The surface roughness of an acrylic denture base is also affected by mechanical or chemical polishing techniques. [26] The rougher surface may be associated with such discoloration, patient discomfort, increased microbial colonization and bio-film formation. [27] Bacteria and fungus have more affinity for adhering to a rough denture material. [28] Therefore, when making a decision for the most appropriate denture base material for a given situation, the surface roughness of each material should be considered.

III. Bio-film

Bio-films are microbial communities encased in a matrix of extracellular polymeric substances (EPS) and display phenotypic features that differ from their planktonic or free-floating counterparts.[14, 29] Dentures, just like natural teeth, can retain calculus, plaque and stains. Denture plaques contain a large number of oral bacteria, fungi and other organisms. There is general consensus that the composition of denture plaque is similar to that of plaque in the dentate patient.[30] Bacteria and fungi within the bio-film can lead to several oral complications, such as denture stomatitis and chronic atrophic candidiasis, as associated with diabetes or HIV. Medically compromised patients are more susceptible to bio-film complications. A mature bio-film is more resistant to antimicrobial agents than an immature bio-film. The microbial colonization of the denture bio-film is very complex and different.

Candida albicans is a diploid fungus that grows both as yeast and as filamentous cells and is a causal agent of opportunistic oral and genital infections in humans[31]. Candida enters newborn infants immediately after birth and its growth is checked by the infant's immune system. It typically remains benign over a life time. *Candida albicans* resides in the mouth, throat,

intestines and genitourinary tract of human bowel flora. Seventy-five percent of healthy adults have *Candida albicans* in the oral cavity. Most common oral fungal infections and dentures stomatitis are associated with Candida [31].

The immune system keeps Candida proliferation under control, but when the immune response is weakened there is an increase in its growth. Candida may colonize the oral cavity of denture-wearing patients many times, especially in the presences of co-factors such as systemic illness or salivary PH[32]. Candida growth can be associated with intraoral environmental changes, for example with an unhygienic prostheses, xerostomia or systemic factors such as diabetes and immunodeficiency. Moreover, it may lead to several systemic conditions, such as pulmonary and gastrointestinal diseases, bacterial endocarditis and general infection of the respiratory tract[33]. Unfortunately, it is considered as the fourth most common cause of hospital-acquired systemic infection in the United States of America with a 50% mortality rate[34, 35]. In brief, *C. albicans* may cause superficial infections such as oral and vaginal candidosis or a life-threatening infection.

There are a large number of studies that have investigated the characteristics of single and mixed species bio-films that consist of *Candida albicans* and various bacteria. [36, 37, 38, 39] The bio-film forming ability was greater for non-*Candida albicans Candida* species than for *C. albicans* species with interspecies variations. The initial stage of oral candidosis is attachment of *C. albicans* to the host surface, such as on denture surfaces. Thrush is the most common form of oral candidosis and is characterized by soft, creamy-colored and elevated plaques. The adherence of *Candidia* to the superficial epithelial cells plays an important role in infection. The fitting

surface of the denture is also considered as a reservoir for pathogens. For this reason, the prevention and management of bio-film will decrease risk of infection.

There are many virulence factors that affect the pathogenicity of Candida; these include [40]:

a. Morphological transition between yeast and hyphal form
b. Expression of adhesion and invasion on cell surface
c. Thigmotropism
d. Formation of bio-film
e. Phenotypic switch
f. Secretion of hydrolytic enzymes.

C. albicans is considered a polymorphic fungus, and it can grow either as ovoid-shaped building yeast, as elongated ellipsoid cells with constrictions at sept or parallel walled true hyphae [41]. It can also be white and opaque cells form during the switch. Environmental changes have an effect on the morphology of *C. albicans*:

a. Low PH < 6 ⟶ Yeast form

b. High PH > 7 ⟶ Hyphal growth

An acidic environment increases colonization of the Candida species. The presence of a low PH is associated with most denture patients, especially those with high sucrose and glucose in their diet (Figure 3). One more important virulence factor is the capacity of *C. albicans* to form a bio-film on biotic or abiotic surfaces. Dentures are considered as abiotic, but the mucosal surface is biotic. The bio-films thus form in variety of processes: [42, 43]

1. Adherence of the yeast to the biotic and abiotic surfaces

2. The proliferation of this yeast to a large number to establish colonization
3. Formation of hyphal cells
4. Accumulation of the extracellular matrix material
5. Dispersion of the yeast cells from bio-film complex which has a direct role in the virulence.

Specific Aims and Hypothesis

The main purpose of the present research was to compare *C. albicans* retention on the surface of seven different denture base materials and its relation to surface roughness. The hypothesis was that a less porous denture base would have significantly lower *C. albicans* counts.

Study Groups and Outcomes

The groups of the study were denture base materials with different processing techniques, including:

- CAD/CAM (Avadent) dentures samples prepared with Lucitone 199 ® (Dentsply, York, PA) under extreme pressure and heat (manufacturer supplied information).
- Eclipse® (Dentsply, York, PA) a light cured denture based material resin.
- Lucitone FRS flexible ® (Dentsply, York, PA).
- SR Ivocap High Impact (Ivoclar Vivadent, Lichtenstein).
- Nature-CRYL Pour acrylic resin (GC America, Alsip, IL).
- DC acrylic S.P
- Clear Ivocap (Ivoclar Vivadent)

The outcome of this study was the percent of *Candida Albicans* adhesion to various denture base materials. The findings of this research may have a direct application in clinical practice and particularly help in improving the oral and general health of medically compromised, high-risk edentulous patients.

Research Design and Methods

I. Research Design

The study was designed to be an experimental *in vitro* study in order to test *C. albicans* retention at the fitting surface of differently-processed denture base materials. This study was conducted at Tufts University School of Dental Medicine (TUSDM) and the Sackler School of Graduate Biomedical Sciences.

II. Research methods

a. Specimen preparation

The material used for fabrication of the denture base samples was a polymethylmethacrylate (PMMA) with different polymerization and fabrication techniques. There was a total of seven groups. All samples were prepared with standard dimensions (5mm x 15mm x 2 mm) to fit with other laboratory equipment for subsequent biologic tests. The surfaces of the samples were finished according to manufacturer recommendations.

A 2 mm thick wax sheet (Hygenic®) was cut with a sharp Bard-Parker Scalpel blade, with a diameter of 15 x 5 mm. This sheet was then adapted to the excavated area of the flat cast with gentle finger pressure after softening it in warm distilled water. The cast was then invested using dental stone to create a good reproduction of the surface. All samples were fabricated with an optimum mix of powder and liquid, and working time and curing time according to

manufacturer's instructions. Any excess was then trimmed from the acrylic samples. Samples were then trimmed to the desired dimension using cutting disk. Both surfaces of the sheets were then finished and polished using standard laboratory techniques (pumice) and denture burs. Finally, samples were trimmed to the desired dimension using a cutting disk.

The Avadent CAD/CAM samples were prepared by milling of the Avadent pack using a milling machine (Figure 4). All the samples were washed and stored in sterile distilled water in order to exert any exercise monomer. Prior to using samples for any laboratory experiments, they were sterilized in an ultraviolet light.

b. Specimen conditioning and culture

Preparation of the frozen glycerol stock of strain SC5314 required several steps. Initially, the Candida was streaked from frozen glycerol and spread on YPD for 2 days at 30°C (Figure 5). Then, a single colony of the *C. albicans* was inoculated and left to grow overnight at 30°C in 3-5 mls YPD. Then one ml of the overnight culture was mixed with 0.5 ml glycerol and frozen at -80°C.

The *C. albicans* suspension was prepared as Streak SC5314 from frozen stock on YPD plates. Then it was incubated at 30°C for 2 days (Figure 6). A single colony was then inoculated into 5 ml CM-ura and incubated at 30°C wheel, overnight.

On the next day, harvested cells were placed in a bench top centrifuge, at 4000 rpm x 6 min, room temperature. Later this was re-suspended and washed with 5 mls PBS and repeated. Suspended cells were then placed in 5 mls PBS (Figures 7,8). Dilution of an aliquot was then done at 1:200 (10 ul + 2 mls PBS). Then CFU was counted with a hemacytometer (Figure 9). CFU/ml was subsequently determined. Finally, this was diluted to 1 x 10^6 CFU/ml in YNB with glucose.

All samples were sterilized with 70% EtOH. Samples were then conditioned by liquid artificial saliva containing buffering agents and cellulose derivatives that were used to moisten samples in order to increase stickiness and moistening. All samples were coated with saliva for 4 h at 37°C.

All QIAcube tubes were filled with two ml of the prepared *C. albicans* suspension. Then the denture base samples were inserted using fine forceps. Attention was given to the sorting of the samples according to its group and sample number. The sample pieces were placed on edge and completely covered by the volume of the suspension. The suspension volume of each sample was recorded. All samples were incubated in the *C. albicans* suspension for 1 hour at 37°C. The *C. albicans* suspension was then aspirated off, leaving only the samples. Samples were then washed twice with PBS in order to remove non-adherent cells. The sample pieces were then

observed by reflecting light microscope to observe adherent cells. The sample together with 1 ml (sterile PBS + 0.1% SDS) was shaken in a Qialyzer for 1 min (Figure 10).

The samples were then observed again under microscope to observe adherent cells. The serial dilutions of the original suspension (10^6 cells/ml) were then plated with the suspension that was removed from the material (Plate 20 ul of 10-2 and 10-3 dilutions). All samples were spread on several glucose agar plates and aerobically incubated at 37°C for 48 h. The spots were spread out with a pipet tip. Finally, the total amount of CFU in each was calculated, including the amounts bound to pieces, and percent of binding.

c. Calculating the total amount of *C.albicans*

The total amount of *C. albicans* was determined by using a plating technique. The total amount of CFU (Colony Forming Unit) in each plate for each group was calculated, including the amounts bound to pieces, and percent of binding (Figure 11).

e. Measurement of surface roughness (Ra)

The surface roughness (Ra), indices of hydrophilicity for all samples were measured using a digital profilometer. The measurements were done with a Mitutoyo profilometer SURFTEST (SJ-201) at the research facility of the Henry M. Goldman School of Dental Medicine at Boston University. The SURFTEST has a wide measurement range (-200μm to +150μm).

Statistical Analysis

Data were analyzed by the Statistical Package for the Social Sciences program (SPSS, Inc, Chicago, IL, USA). A one-way ANOVA was not used to test the significance of the *C. albicans* adhesion to the samples or surface roughness because the histograms did not look like the normal curve. The Kruskal-Wallis test was used instead, with Mann-Whitney U tests and Bonferroni correction for post-hoc comparisons. The correlation between surface roughness and *C. albicans* adhesion was analyzed via the Spearman correlation.

A power calculation was conducted using nQuery Advisor (Version 7.0). Assuming means of 6.3% (Eclipse), 1.3% (Ivocap), 1.1% (FRS), 4.9% (Clear Ivocap), 1.7% (DC Acrylic s.p.), 1.7% (Nature Pure acrylic resin), and 0.8% (Avadent Luceton), as well as a common standard deviation of 3.7 across groups,* a sample size of n=15 per group was adequate to obtain a Type I error rate of 5% and a power greater than 99%.

* Results from pilot study

Results

Candida Albican adhesion values are described in Table 1 as medians and inter-quartile ranges (IQR). The medians ranged from 0.0% for Avadent to 8.0% for Eclipse. Figure 11 shows side-by-side boxplots of the adhesion values. Overall, Eclipse, Clear Ivocap and Ivocap showed more adhesion than the FRS, DC, Nature GC and Avadent groups. The results of the Kruskal-Wallis test were statistically significant ($p<0.001$). Groups exhibiting significant differences are shown in Table 1; groups sharing the same letter are not significant.

Surface roughness (Ra) values are summarized in Table 2 as medians and IQR values. The medians ranged from 0.03 µm to 0.70 µm. Figure 12 shows side-by-side boxplots of the surface roughness values. Overall, FRS and Eclipse showed rougher surfaces than Clear Ivocap, Ivocap, Nature GC and Avadent. The results of the Kruskal-Wallis test were statistically significant ($p<0.001$). Groups with significant differences are shown in Table 2.

Table 3 summarizes the results of the Spearman correlation analyses between individual surface roughness (Ra) and *C. albicans* adhesion. Correlation values ranged from 0.603 to 0.961. A statistically significant correlation was found between surface roughness and *C. albicans* adhesion for every group.

Discussion

The adhesion of micro-organisms to denture surfaces is related to surface free energy and roughness of the denture material.[44] In this research, an *in vitro* study was developed to compare the adhesion of *C. albicans* to seven different types of denture base materials in terms of surface roughness. The findings suggest that the surface roughness of a denture material is associated with the degree of Candida adhesion.

C. albicans adhesion has four phases, which include: transport to the surface, initial adhesion, attachment and colonization.[18] Additionally, the formation of the Candida biofilm on PMMA strips occurs in three phases: early phase (0–11 h), intermediate phase (12–30 h), and a maturation phase (38–72 h). The adhesion of the *C. albicans* confirmed by study were done with 16 different spices of *C. albicans* and showed significant adhesion for all groups.[45]

In the present study, *C. albicans* suspension was prepared as Streak SC5314, then incubated at 30°C for 2 days. Samples were then incubated for one hour on *C. albicans* suspension. The Candida suspension that was removed from the material was spread on several glucose agar plates and aerobically incubated at 37°C for 48 h. Then, CFU (Colony Forming Unit) in each plate for each group was calculated, including the amounts bound to pieces, and % binding.

The data of this research indicated that Avadent samples showed the lowest adhesion percent in comparison to other samples. The Nature-CRYL Pour acrylic resin denture base resin showed low adhesion values, but greater than the CAD/CAM denture base material. This was followed by Lucitone FRS flexible denture base resin, followed by DC denture base, and then followed by Ivocap. Conversely, a significant high adherence was found in Eclipse denture base resin and the Clear Ivocap denture base material, but the Eclipse showed more adhesion. Most of the *C. albicans* adhesion percentages fell between 0% and 8% of adhesion among the study groups. *C. albicans* adhesion showing significant differences among different resin materials has been reported by other investigators such as Minagi et al.,[46] Klotz et al.,[47], Waters et al.[44] and Radford.[18]

It was clear that surface roughness is related to the formation and colonization of *C. albicans* on the denture surface. The rougher surface means a greater available surface area of active sites for a thermodynamic reaction.[48] In this research, the surface roughness of seven denture base materials was measured, because it is a factor for the retention of the micro-organisms on surfaces.[49] The denture material surfaces were finished using the same technique, and significant differences in the surface roughness values (Ra) were detected (Table 2). Additionally, a statistically significant correlation between the Ra values and *C. albicans* adhesion was detected by the Spearman correlation analysis (Table 3). This finding suggested that surface roughness within a range > 0.07 μm plays an important role in high *C. albicans* adhesion. This may occur as a result of the effect of the contact angle, which implicates surface energy and surface roughness.[50]

The research has some limitations. The influence of human saliva on *C. albicans* adhesion to the materials was not observed. The study used artificial saliva as a co-factor that improved adhesion in the study sample. The use of a confocal light microscope was recommended to confirm the plating techniques results. The effect of antifungal treatments was not applied to the different samples due to limited access in the laboratory. Time and equipment factors may affect the addition of other tests to different materials.

Conclusion

A less porous denture base material has a significantly lower *C. albicans* count. The Avadent samples showed the lowest adhesion percent in comparison to other samples. The Nature-CRYL Pour acrylic resin denture base resin showed low adhesion values, but greater than the CAD/CAM denture base material. This was followed by Lucitone FRS, a flexible denture base resin, followed by DC denture base, and then followed by Ivocap. Conversely, a significantly high adherence was found in Eclipse denture base resin and the Clear Ivocap denture base material, but the Eclipse showed more adhesion. A significant correlation was found between the adhesion of Candida and the surface roughness of the denture base material.

List of Figures:

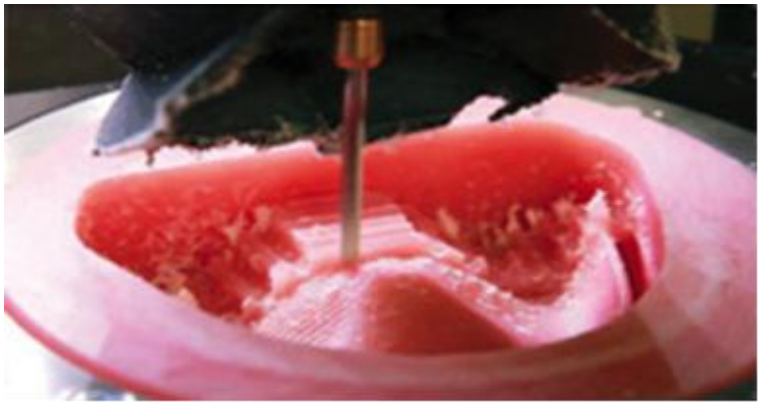

Figure 1. Subtractive denture base preparation technique using CAD/CAM

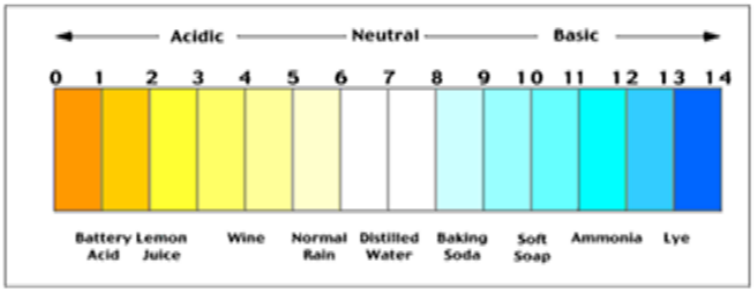

Figure 2. PH and relation to patient oral health

Figure 3. Avadent CAD/CAM pack during milling process

Figure 4. The frozen glycerol stock

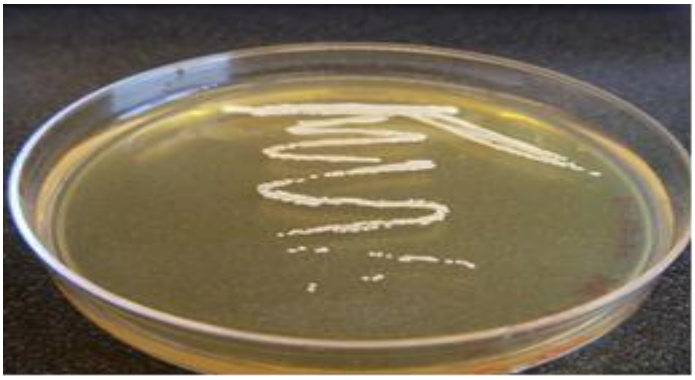

Figure 5.Incubated *C.albicans*

Figure 6. Benchtop centrifuge, 4000 rpm x 6 min

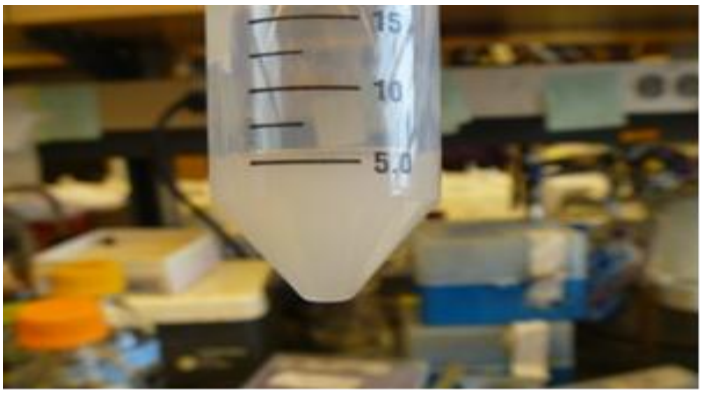

Figure 7. Diluted *C.albican* suspension

Figure 8. Hemacytometer

Figure 9. Qialyzer

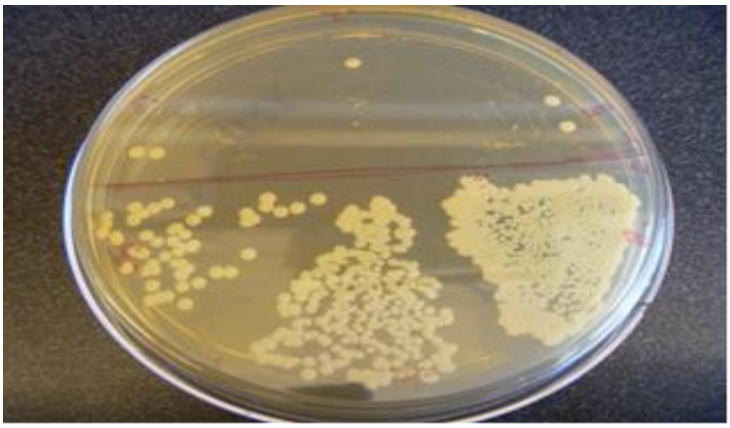

Figure 10. The growing *C.albicans*

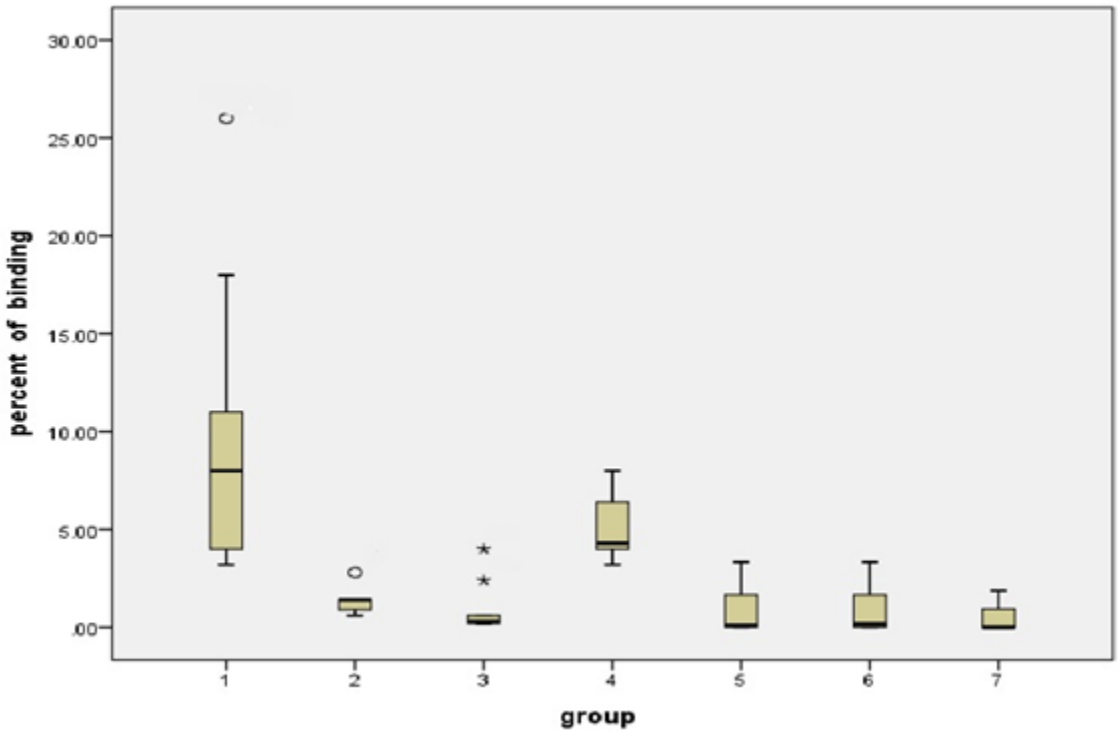

Figure 11. Percent of *C.albican*s binding for each study group

1Eclipse, 2Ivocap, 3 FRS, 4 Clear Ivocap, 5 DC, 6 Nature GC, 7 Avadent

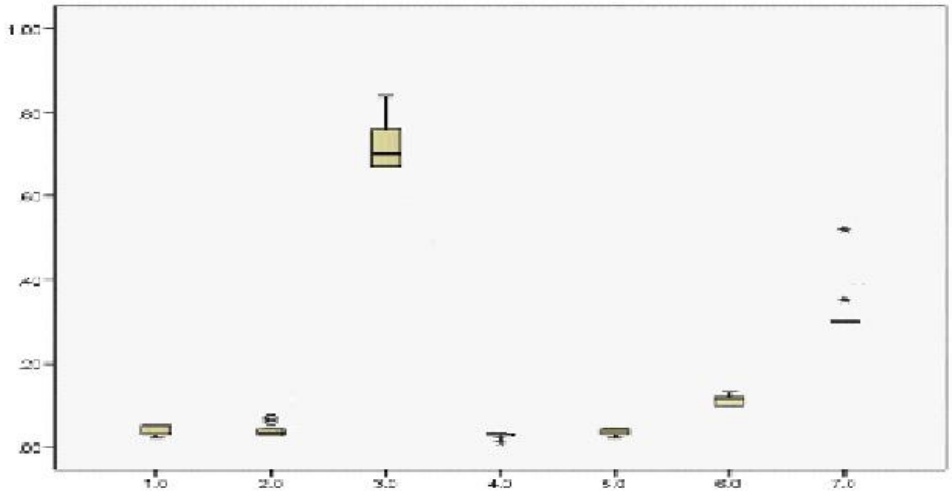

Figure 12. Surface roughness (Ra) for each study group

1Eclipse, 2 FRS, 3 Ivocap, 4 Clear Ivocap, 5 DC, 6 Nature GC, 7 Avadent

List of Tables :

Group	N	Median	Interquartile Range	p
Eclipse	15	8.0[a]	7.5	<0.001
Ivocap	15	1.4[c,d,e]	0.6	
FRS	15	0.3[b]	0.4	
Clear Ivocap	15	4.3[a]	2.4	
DC	15	0.1[b,c,f]	1.7	
Nature GC	15	0.2[f,g,d]	1.7	
Avadent	15	0.0[e.g]	1.1	

Table 1**.** *C.albicans* adhesion percent for each study group

Group	N	Median	IQR	P
Eclipse	15	0.05[a,b,c,d,e]	0.02	<0.001
Ivocap	15	0.03[a,f,g,h,j]	0.01	
FRS	15	0.70[a,f,k]	0.09	
Clear Ivocap	15	0.03[i]	0.00	
DC	15	0.04[c,g,k,l]	0.01	
Nature GC	15	0.12[l,h,d]	0.02	
Avadent	15	0.03[j,e]	0.00	

Table 2. Surface roughness (Ra) for each study group

Group	N	Spearman's correlation	P
Eclipse	15	0.936	<0.001
FRS	15	0.843	<0.001
Ivocap	15	0.906	<0.001
Clear Ivocap	15	0.758	0.001
DC	15	0.603	0.017
Nature GC	15	0.961	<0.001
Avadent	15	0.880	<0.001

Table 3. Correlation between surface roughness and percent binding of *C. albicans*

References

1. Paul E P, Denis B, Hiroshi O, Saskia E, Charlotte N. The global burden of oral disease and risk to oral health. Bulletin of world health organization. 2005; 83: 661-669.
2. Douglass CW, Shih A, Ostry L. Will there be a need for complete dentures in the United States in 2020? J Prosthet Dent. 2002 Jan; 87(1):5-8.
3. Douglass CW, Gammon MD, Atwood DA. Need and effective demand for prosthodontic treatment. A report: Part one. Oral Health. 1988 Nov;78(11):11-7, 21-3.
4. Enwonwu CO, Phillips RS, Ferrell CD. Temporal relationship between the occurrence of fresh noma and the timing of linear growth retardation in Nigerian children. Trop Med Int Health. 2005 Jan; 10(1):65-73.
5. Felton D, Cooper L, Duqum I, Minsley G, Guckes A, Haug S, Meredith P, Solie C, Avery D, Deal Chandler N; American College of Prosthodontists. Evidence-based guidelines for the care and maintenance of complete dentures: a publication of the American College of Prosthodontists. J Prosthodont. 2011 Feb; 20 Suppl 1:S1-S12.
6. US Department of Health. Health Care Financing Administration. Available at: URL: http://www.nidr.nih.gov./sgr/sgrohwed/toc.htm. Accessed at Mar 2, 2013.
7. Douglass CW, Gammon MD, Atwood DA. Need and effective demand for prosthodontic treatment. A report: Part one. Oral Health. 1988 Nov;78(11):11-7, 21-3.
8. Felton DA. Edentulism and comorbid factors. Tex Dent J. 2010 Apr;127(4):389-401.
9. Marcos a Silva, Rafael L, Guilherme J, Lucas A, Jose M. Association between implants and removable partial dentures: review of the literature. RSBO. 2011 Jan-Mar; 8(1): 88-92.
10. Peyton FA. History of resins in dentistry. Dent Clin North Am 1975; 19: 211-222.
11. Tihan TG, Ionita MD, Popescu RG, Iordachescu D. Effect of hydrophilic-hydrophobic balance on biocompatibility of poly(methylmethacrylate) (PMMA)-hydroxyapatite (HA) composites. Mater Chem Phys 2009; 118: 265-269.
12. Cheng YY, Cheung WL, Chow TW. Strain analysis of maxillary complete denture with three-dimensional finite element method. J Prosthet Dent 2010; 103:309-318.
13. Beyli MS, Von Fraunhofer. An analysis of causes of fracture of acrylic resin dentures. J Prosthet Dent 1981; 46: 238-241.

14. Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM. Microbial biofilms. Annu Rev Microbiol. 1995; 49:711-45. Review.
15. Chopde N, Jawale B, Pharande A, Chaudhari L, Hiremath V, Redasani R. Microbial colonization and their relation with potential cofactors in patients with denture stomatitis. J Contemp Dent Pract. 2012 Jul 1;13(4):456-9.
16. Epstein JB. Antifungal therapy in oropharyngeal mycotic infections. Oral Surg Oral Med Oral Pathol. 1990 Jan;69(1):32-41. Review.
17. Arendorf TM, Walker DM. Denture stomatitis: a review. J Oral Rehabil. 1987 May;14(3):217-27. Review.
18. Radford DR, Challacombe SJ, Walter JD. Denture plaque and adherence of Candida albicans to denture-base materials in vivo and in vitro. Crit Rev Oral Biol Med. 1999;10(1):99-116. Review.
19. Webb BC, Thomas CJ, Willcox MD, Harty DW, Knox KW. Candida-associated denture stomatitis. Aetiology and management: a review. Part 1. Factors influencing distribution of Candida species in the oral cavity. Aust Dent J. 1998 Feb;43(1):45-50. Review.
20. Tyldesley, Anne Field, Lesley Longman in collaboration with William R. (2003). Tyldesley's Oral medicine (5th ed. ed.). Oxford: Oxford University Press. pp. 35–40. ISBN 0192631470.
21. Abuzar MA, Bellur S, Duong N, Kim BB, Lu P, Palfreyman N, Surendran D, Tran VT. Evaluating surface roughness of a polyamide denture base material in comparison with poly (methyl methacrylate). J Oral Sci. 2010 Dec;52(4):577-81.
22. Jefferies SR. Abrasive finishing and polishing in restorative dentistry: a state-of-the-art review. Dent Clin North Am. 2007 Apr;51(2):379-97, ix. Review.
23. Kuhar M, Funduk N. Effects of polishing techniques on the surface roughness of acrylic denture base resins. J Prosthet Dent. 2005 Jan;93(1):76-85.
24. Quirynen M, Marechal M, Busscher HJ, Weerkamp AH, Darius PL, van Steenberghe D. The influence of surface free energy and surface roughness on early plaque formation. An in vivo study in man. J Clin Periodontol. 1990 Mar;17(3):138-44.
25. Tucker TN. Allergy to acrylic resin denture base. J Prosthet Dent. 1981 Dec;46(6):602.

26. Basker RM, Hunter AM, Highet AS. A severe asthmatic reaction to poly(methyl methacrylate) denture base resin. Br Dent J. 1990 Oct 20;169(8):250-1.
27. Jagger DC, Harrison A, Jandt KD. The reinforcement of dentures. J Oral Rehabil. 1999 Mar;26(3):185-94. Review.
28. John J, Gangadhar SA, Shah I. Flexural strength of heat-polymerized polymethyl methacrylate denture resin reinforced with glass, aramid, or nylon fibers. J Prosthet Dent. 2001 Oct;86(4):424-7.
29. Theilade E, Budtz-Jørgensen E. Predominant cultivable microflora of plaque on removable dentures in patients with denture-induced stomatitis. Oral Microbiol Immunol. 1988 Mar;3(1):8-13.
30. Ruhnke M. Skin and mucous membrane infections. In: Calderone RA, ed. Candida and Candidiasis: ASM Press, Washington, DC, pp. 307-325., 2002.
31. Chopde N, Jawale B, Pharande A, Chaudhari L, Hiremath V, Redasani R. Microbial colonization and their relation with potential cofactors in patients with denture stomatitis. J Contemp Dent Pract. 2012 Jul 1;13(4):456-9.
32. Silva-Lovato CH, Totti AM, Paranhos HF, Totti VG. Evaluation of a computerized method for denture biofilm quantification: inter-examiner reproducibility. J Prosthodont. 2009 Jun;18(4):332-6.
33. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007 Jan;20(1):133-63.
34. Pfaller MA, Diekema DJ. Epidemiology of invasive mycoses in North America. Crit Rev Microbiol. 2010;36(1):1-53
35. Adam B, Baillie GS, Douglas LJ. Mixed species biofilms of Candida albicans and Staphylococcus epidermidis. J Med Microbiol. 2002 Apr;51(4):344-9.
36. Baillie GS, Douglas LJ. Candida biofilms and their susceptibility to antifungal agents. Methods Enzymol. 1999;310:644-56
37. Henriqus M, Azerdo J, Oliviera R. Candida and Candida dubliniesis: comparison of biofilm formation in term of biomass and activity. Br J Biomed Sci. 2006; 63:5-11.
38. Jin YY, Yip HK, Samaranyka YH, Yau JH. Bio-film forming ability of Candida albicans and Candida parapsilosis on bioprosthatic surfaces. Infect Immun 2002; 70:878-88.

39. Penha SS, Birman EG, Silviria FR, Paula CR. Frequency and enzymatic activity (protinase and phospholipids) of Candida albicans from edentulous patients, with or without denture stomatitis. Pesq Odontol Bras. 2000; 14:119-122.
40. Berman J, Sudbery PE. Candida Albicans: a molecular revolution built on lessons from budding yeast. Nat Rev Genet 2002; 3:918-30.
41. Sudbery P, Gow N, Berman J. The distinct morphogenic states of Candida albicans. Trends Microbiol. 2004 Jul;12(7):317-24.
42. Finkel JS, Mitchell AP. Genetic control of Candida albicans biofilm development. Nat Rev Microbiol. 2011 Feb;9(2):109-18.
43. Muzyka BC. Oral fungal infections. Dent Clin North Am 2005; 49:49–65.
44. Waters MG, Williams DW, Jagger RG, Lewis MA. Adherence of Candida albicans to experimental denture soft lining materials. J Prosthet Dent 1997;77:306–12.
45. Chandra J, Kuhn DM, Mukherjee PK, Hoyer LL, McCormick T, Ghannoum MA. Biofilm formation by the fungal pathogen Candida albicans: development, architecture, and drug resistance. J Bacteriol 2001;183:5385–94.
46. Minagi S, Miyake Y, Inagaki K, Tsuru H, Suginaka H. Hydrophobic interaction in Candida albicans and Candida tropicalis adherence to various denture base resin materials. Infect Immun. 1985 January; 47(1): 11–14.
47. Klotz A, Drutz D, Zajic J. Factors governing adherence of Candida species to plastic surfaces. Infect Immun. 1985 October; 50(1): 97–101.
48. Giraldez MJ, Resua CG, Lira M, Oliveira ME, Magariños B, Toranzo AE, et al. Contact lens hydrophobicity and roughness effects on bacterial adhesion. Optom Vis Sci 2010;87:E42631.
49. Verran J, Maryan CJ. Retention of Candida albicans on acrylic resin and silicone of different surface topography. J Prosthet Dent 1997;77:535–9.
50. Busscher HJ, van Pelt AWJ, de Boer P, de Jong HP, Arends J. The effect of surface roughening of polymers on measured contact angles of liquids. Colloids Surf 1984;9:319–31.