Effect of Microwave Cured Acrylic Resin on Candidal Growth in Complete denture.

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Introduction

Loss of teeth is accompanied by adverse esthetic and biomechanical sequelae. This predicament is worst when entire periodontal ligament support is lost and the patient becomes completely edentulous.

These problems are accompanied by reduction of the person ability for mastication and therefore disturbed nutritional state accompanied with defective speech and bad esthetics. Many patients seek a solution for their problems and the conventional treatment for these cases is the construction of complete denture\(^{(1,2)}\).

Since 1937, heat-cured Polymethyl methacrylate is the most commonly used complete denture base material due to its excellent appearance, easy in processing and repair\(^{(3)}\).

However, the use of heat-cured acrylic resin denture base has been associated with many problems, such as the liabilities to breakage during service, hypersensitivity reaction as well as accumulation of denture plaque. The porous surface texture of heat-cured acrylic resin favors the accumulation of dental plaque and creates an environment for Candidal colonization that irritates the denture bearing area\(^{(3)}\).

Several types of modified polymethyl methacrylate have been introduced for denture base construction. These include
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self cured resins, pour type resins, high impact strength resins, light- cured resins and microwave- cured resins (4).

Acrylic resin is traditionally polymerized with a water bath method. Microwave energy polymerization, was first reported as an alternative method.

Microwaves are a form of electromagnetic radiation used mainly in radar and telecommunications. Microwaves were used as means of curing, and are generated by a magnetron and travelled in a straight line along the wave guide in what is called the dominant mode (5, 6).

Microwave-cured denture base are characterized by their accurate fit and reduction in the external and internal porosity of the denture base. Acrylic resin polymerized by microwave is highly accepted since it is more resistant to mechanical failure than conventionally heat-cured acrylic resin (7).

Candida species are normal oral commensals present in 17% to 60 % of apparently healthy persons (8).

Multiple factors have been implicated to predispose to Candidal growth and colonization. Wearing of dentures is among the most important risk factors affecting Candidal carriage. The presence of an intraoral appliance produced alternation in the ecological environment of the prosthesis-
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covered area. Such ecological changes promotes colonization and adhesion of *Candida albicans* (*C. albicans*) which was found to nourish in denture wearers, as the denture act as a reservoir and provide a suitable environment for growth and adhesion of Candida. This causes irritation of the oral mucosa due to concentration of exotoxins and metabolic product of fungal colonies\(^9,10\).

Awareness of the susceptibility of the denture base to *Candida albicans* colonization should be an important factor in their use to preserve and maintain the health of the oral mucosa. Accordingly, this study was prompted to evaluate and compare the effect of conventional heat-cured and microwave-cured acrylic denture resin on Candidal growth.
I-REVIEW OF LITERATURE

I.1. Denture base material:

The denture base is “that part of the denture that rests on the foundation tissues and to which teeth are attached” \(^{(11)}\).

Individual denture bases are either metallic denture base constructed either from precious alloys (gold), non-precious metallic alloys (cobalt chromium or Nickel chromium) or non-metallic denture base that are constructed from polymers. Such polymers are chosen based on their availability, strength and durability, satisfactory thermal properties, processing accuracy, dimensional and chemical stability, color stability, insolubility and low sorption in oral fluids, absence of taste and odour, biocompatibility, natural appearance, adhesion to plastics, metals and porcelain, and easy of fabrication and repair \(^{(12)}\).

Since there is no denture base materials that satisfies all the above mentioned characteristics and requirements, various materials have been used to construct denture base which includes acrylic denture base materials which were found to form 99% of all fabricated complete dentures \(^{(13)}\).

Acrylic resin polymer was introduced as a denture base material in 1937. It is principally formed of polymethylmethacrylate. Previously, materials such as vulcanite nitrocellulose, phenol-formaldehyde and vinyl plastics were used for construction of denture base \(^{(14)}\).
Although PMMA based resins are not ideal in every respect, the combination of properties such as working characteristics, minimum expense, excellent esthetics, accuracy of fit, stability in the oral environment, and ease of processing account for their popularity and universal use (15).

Dimensional instability, which is either due to polymerization or water sorption, is principal disadvantage of acrylic resin base. Shrinkage is particularly noticeable in the posterior border region, where the retentive seal and stability of the prosthesis can be compromised. Also, absorption of water by acrylic resin is of great importance since it will be accompanied by dimensional changes (16).

Various polymers have been developed for use as denture base resins to overcome some of the mechanical deficiencies of polymethymethacrylate such as nylon, epoxy resin, vinyl acrylic and polycarbonate (17).

The chief advantages of nylon denture are strength and lightness, in addition to these advantages, nylon could be used also in cases susceptible to inflammation due to lack of residual monomer in contrast to conventional denture base material, however, nylon suffered from some undesirable properties that limits it’s wide application as stains, great flexibility that could stiffen by incorporation of glass spheres, become rough after few weeks in mouth, thus encourages bacterial growth (18, 19).

Epoxy polymers are not used because of toxicity of some curing
agents present in its ingredient \(^{(15)}\).

A denture constructed from polyvinyl acrylic will deform elastically to greater extent under forces of mastication than comparable polymethyl methacrylate. It has an impact strength which is twice that of polymethyl methacrylate which indicates that vinyl acrylic absorbs more energy on impact and is more resistant to fracture \(^{(20)}\).

Polycarbonate needs injection moulding technique which necessitates the use of specialized apparatus \(^{(21)}\).

I.1.1. Types of Acrylic resin polymer:

Several types of modified polymethylmethacrylate have been introduced for denture base applications. The details of these types are as follows:

I.1.1.1. Conventional heat-cured PMMA:-

The polymerization of this resin is an additional reaction that requires activation of an initiator, such as benzoil peroxide to produce free radicals. The polymerization process occurs when the free radicals open the double bonds of the methylmethacrylate creating a Chain reaction in which the monomers attach to the polymer free radicals \(^{(22)}\).

This material is supplied as a powder and liquid. The powder is beads of polymethylmethacrylate (PMMA) polymer or copolymer, benzoil peroxide initiator, pigments, dyes, opacifiers and plasticizer liquid is
methylmethacrylate (MMA) monomer with a cross-linking agent (usually 5%-15% glycol dimethacrylate) and a small amount of inhibitor (hydroquinone) to avoid premature polymerization and enhances shelf life. The cross-linking molecules are added to reduce small surface cracks which forms in the denture when it is allowed to dry. These small cracks are called craze cracks and are produced by stresses created during drying \(^{(23)}\).

Heat cured acrylic denture base material is characterized by being non-toxic, insoluble and inert in oral fluids. It is esthetically accepted, easy to process and capable to repair with simple equipment, it is also inexpensive and has a satisfactory shelf life \(^{(24, 25)}\).

Its tensile strength is approximately 50 MegaPascal and its compressive strength approximately 76 MegaPascal. When these are combined with lack of fracture toughness, it is perhaps surprising that acrylic resin dentures are prone to fracture \(^{(26, 27)}\).

The unpolymerized monomers, remaining after curing, results in plasticizing action, which weakens and softens the material \(^{(28)}\).

Conventional acrylic resin shows some disadvantages such as tissue hypersensitivity due to its high residual monomer content that leaches out within 17 hours causing tissue irritation, dimensional instability which is either due to polymerization shrinkage or water sorption and porosity which affects the strength of the material especially against sudden drop \(^{(29)}\).
I.1.1.2. Chemically activated denture base resins:

Chemically activated resin is known as cold cured resin or self cured resin. The material contains a chemical activator, which activates benzoil peroxide so that polymerization can be completed at room temperature. However the degree of polymerization achieved by the use of a chemical activator is not as high as activation by heat. Also, color stability of the chemically activated resin is inferior to that of heat-cured resin (30).

The higher residual monomer acts as a plasticizer, which results in lower transverse strengths. However, the chemically cured acrylic is nearly as hard as the heat cured type if after 2.5 Hours of curing at room temperature, the flask is boiled for 0.5 to 1 hour. Properties comparable to the heat-cured type are obtained and the residual monomer content is considerably reduced (20).

“Provac” was evaluated as a new denture base resin, which is methylmethacrylate cured with the aid of barbituric acid. Provac showed dimensional accuracy better than that of conventional heat curing materials. However, the residual monomer levels in provac was always higher than that obtained from conventional heat cured materials (31).

I.1.1.3. Pour type denture resins:

The chemical composition of the pour type denture resin is similar to the polymethylmethacrylate material that is polymerized at room temperature. The principal difference is in the size of the polymer powder
or beads.

The pour type denture resin is commonly referred to as fluids resin that have much smaller powder particles, when mixed with monomer, the resulting slurry is very fluid. The mix is quickly poured into an agar-hydrocolloid or modified plaster mold and allows polymerizing under pressure at 0.14 MegaPascal(MPa). Centrifugal casting or injection moldings are techniques used to inject slurry into the mold (32).

Dentures fabricated by this technique are less accurate than conventional heat-cured acrylic dentures because of their higher polymerization shrinkage (20).

Pour type acrylics are characterized by lower impact and fatigue strengths, higher creep values, low transverse bond strength, lower water sorption values and higher solubility compared to conventional heat cured acrylic resin (20).

A study on the effect of variation in powder particle size on the manipulation time and mechanical properties of acrylic resin revealed insignificant differences in the tested specimens (33).

**I.1.1.4. Rapid heat polymerized resins:-**

Rapid heat-polymerized resins are hybrid acrylics that are polymerized in boiling water immediately after being packed into a denture flask. The initiator is formulated from both chemical and heat-activated initiators to allow rapid polymerization without porosity; the water is
brought back to a full boil for 20 minutes. After bench cooling at room
temperature, the denture is deflasked, trimmed, and polished in the
conventional manner (32).

The rapid heat cured denture base material has residual monomer
level 1.3% when they are processed for 1 hour in boiling water however if
they are processed for 7 hour at 70 C° and then boiled for 3 hours, the
residual monomer content may be less than 0.4% (20).

The effect of different temperature and curing times of several
commercial acrylic resins was investigated; the materials used were rapid
heat cured, injection molded and two auto-polymerizing resins. It was
found that keeping the temperature of water bath constant through the
curing cycle especially of the auto-polymerizing resins is very important for
obtaining relatively porosity free resin (34).

**I.1.1.5. High impact strength resin:-**

Denture base materials having greater impact strength have been
recently introduced. These polymers are re-inforced with butadiene –
styrene rubber. The rubber particles are grafted to methylmethacrylate to
bond to the acrylic matrix. These materials are supplied in a powder- liquid
form and are processed in the same way as other heat-accelerated
methacrylate resins (35).

**I.1.1.6. Light activated denture base resin:-**

This denture base material consists of urethane dimethacrylate
matrix with an acrylic co-polymer micro-fine silica filler and photo-initiator system. This type of resin needs a high curing unit with special wavelength\textsuperscript{(20)}.

It is supplied in pre-mixed sheets having clay like consistency. The denture base material is adapted to the cast while it is still pliable. The denture base can be polymerized in a light chamber without teeth and used as a record base. The teeth are processed to the base with additional material and the anatomy is sculptured while the material is still plastic. The acrylic is polymerized in a light chamber with blue light of 400 to 500 nm. The denture rotates in the chamber to provide uniform exposure to the light source\textsuperscript{(36)}.

This system eliminates the need for flask, wax, boil-out tanks, packing presses and heat processing units, also to save time in both the dental office and laboratory\textsuperscript{(20)}.

Light activated material was compared to conventional heat cured materials and it was found that they have low elastic modulus and thus they expected to deform elastically to greater extent than conventional heat cured-denture under the forces of mastication. Also since light activated materials contain no methylmethacrylate monomer, therefore they are better in those patients who have sensitivity. Also as a consequence of the high-molecular weight oligomers used in light activated systems, therefore polymerization shrinkage is smaller about 3\% rather than 6 \% shrinkage found in conventional systems, therefore denture base processed by visible light fit better than conventional heat cured resins\textsuperscript{(37)}.
“Triad” is a new visible light cured resin characterized by being non-toxic, bio-compatible, superior strength and show complete polymerization without residual monomer, beside of ease of fabrication, accuracy of fit, patient acceptance and low bacterial adherence which made this material more accepted by dentists \(^{(38)}\).

\textbf{I.1.1.7. Microwave denture bases:-}

\textbf{Microwave radiation:-}

Microwave occupies the portion of the electromagnetic spectrum extending from the frequency of 300 megahertz (MHz) to 300,000 MHz most commercial microwave ovens operate at 2450 MHz \(^{(39, 40)}\).

Microwave energy generated in a magnetron oscillator, was transferred to a heating chamber where the flask with resin was irradiated. During irradiation the microwave energy is absorbed by the object irradiated and changed into heat (dielectric heating). The difference between the ordinary conduction heat and dielectric heat is that with dielectric method, the inside and outside of the substance are equally heated and the temperature rises much more quickly \(^{(41)}\).

Acrylic polymers were first introduced as denture base materials in 1937. Polymethylmethacrylate has been the most commonly resin used in removable complete and partial dentures.

Water- bath curing unit is the most commonly used to process polymethylmethacrylate. Processing with dry heat, steam, infrared,
induction or dielectric heating has also been used. Microwaves are an important addition to this list.\(^{(41)}\)

Kimura et al.,\(^{(42)}\) began a series of studies in the use of microwave as a possible heat source to polymerize denture acrylic resin. Later, Reitz et al., Declerk\(^{(43)}\) and Takamata et al.,\(^{(44)}\) reported significant uses of microwave technique of heat polymerization.

Shlosberg et al.,\(^{(45)}\) demonstrated that microwave polymerization of polymethylmethacrylate denture base resin can be successfully with metal removable partial denture frameworks.

Polymerization by microwave energy shows improvement of adaptation of processed bases which results from homogenous heating of investing plaster and resin by microwave causing few internal stresses to be introduced into processed denture\(^{(46)}\).

Additional advantage which includes shortened dough-forming time, more homogenous dough, a shorter curing time and minimal color changes in the resin are reported\(^{(47,48)}\).

Microwave processing of denture bases is also cleaner and more time efficient\(^{(49)}\). Microwave activated denture base resin are reported to have better dimensional accuracy than that of conventional materials\(^{(50)}\).

However, no difference in the hardness and transverse strength was reported comparing strips of resins cured by microwave to those cured by conventional\(^{(51)}\).
Microwave technique, makes it possible to process resins of various thicknesses in a short period of time and to be confident of the dimensional accuracy of the procedures, with the reduction of the time needed for laboratory procedures, some services, such as relining and rebasing, can be done within a matter of several hours\(^{(52)}\).

The American dental association specification showed that acrylic resin cured by microwave energy is more resistant to mechanical failure than conventionally cured acrylic resin\(^{(53)}\).

**1.2. Bio-compatibility of denture base resins:**

The term "Biocompatibility" is defined in the Donald’s illustrated medical dictionary as being “Harmonious with life and not having toxic or injurious effects on biological function”. Biocompatibility is measured on the bases of localized cytotoxicity such as pulp and mucosal response, systemic responses, allergenicity and carcinogenicity\(^{(54)}\). Craig also defined it as ”The compatibility of manufactured materials and devices with body tissues and fluids”\(^{(55)}\).

Interactions of materials with tissues may alter the normal metabolism and physiological processes. These interactions may be physical or chemical, with cells going through stage of degeneration, death and necrosis. There are three stages: Injury to cells and tissues by any agent which include the biochemical lesion, functional lesion and morphological lesion. An example of injury is an infarct of tissue resulting from ischemia. A reduced oxygen supply to individual cells results in suppression of
Review of Literature

oxidative phosphorylation and adenosine triphosphate production within seconds to minutes (biochemical lesion) followed by decreased function of the sodium pump of the cell membrane. Failure of the pump results in cellular swelling from retention of sodium and water and reduced function (functional lesion), finally, loss in the integrity of the cell and nuclear membranes and release of lysosomal enzymes (morphological lesion). These chemical and physical injuries lead to further connective tissues changes classified as inflammatory reactions, immunological reactions and repair. Based on these criteria, Stanely (56). Advocated the requirements of dental material biocompatibility which are:

- It should not be harmful to the pulp and soft tissues.

- It should not contain toxic diffusible substances that can be released and absorbed into the circularity system to cause a systemic toxic response.

- It should be free from potentially sensitizing agents that are likely to cause an allergic response.

- It should have no carcinogenic potential.

I.3. Adverse reactions to denture base resins:

Acrylic resin denture has the potential to elicit irritation, inflammation, and an allergic response of the mucosa. A study was conducted on the cytotoxicity of substances leachable from acrylic resins, their cultured cells, and means of reducing their leaching. In vivo and in vitro conditions, formaldehyde and methyl methacrylate were significantly
leached into human Saliva and saliva – substitute buffer, especially from autopolymerized resins. Both leachable substances showed cytotoxic potentials in the range of their leaching concentrations. Formaldehyde was cytotoxic at lower concentrations than methylmethacrylate \(^{(57)}\).

In an attempt to decrease the amount of residual monomer in the denture and its release into saliva, immersion of dentures either in hot water \(50^\circ C\) for one hour before insertion or in water \(37^\circ C\) for one to two days before its insertion especially for the autopolymerizing acrylic resin used for rebasing or relining denture bases has been recommended \(^{(58)}\).

Formaldehyde was found to be responsible for allergic inflammation in acrylic denture wearers. Flow injection analysis was developed to quantify the formaldehyde leached from acrylic resin. Different resins were immersed in aqueous solvents at \(37^\circ C\) and the immersion solutions were directly injected into the flow system, in which formaldehyde was detected. Under the optimized conditions, the leached formaldehyde could be quantified in a short time (within 4 min) with high sensitivity and high specificity. In leaching experiments, significant amount of formaldehyde were leached from autopolymerized resins, but not from heat cured and microwave polymerized resins \(^{(59)}\).

Denture sore mouth caused by allergy to the denture material was investigated; the result showed that the residual monomer was the allergen. Patch test of the skin with drillings from upper and lower denture made of a heat-polymerized methylmethacrylate resin was carried out, only that surface of the upper denture that is in contact with the hard palate was
allergenic, all other surfaces of the upper denture as well as the complete lower denture were non-allergenic (60).

A new technique made by a re-polymerization comprising initial application of heat centrally in the flask, including tin foiling of the palatal half of the mould, a check up 18 months later showed no stomatitis and the retention and stability of the denture were very satisfactory, they concluded that the mode of polymerization affects the allergenic properties of acrylic denture base resins (61).

The oral mucosa was found to be damaged by denture bases containing 1.7, 2.5 and 3.2% residual monomer. These levels are 6-11 times greater than the normal baseline value for dentures produced by along curing cycle (0.3%). A short curing cycle produced dentures that are more likely to induce mucosal reactions than dentures cured by a long curing cycle (61).

Contact sensitization is especially caused by be resin itself; plasticizers, fillers and pigments can occasionally be responsible. Since completely cured resins are not sensitizers, resin sensitization is always due to the presence of uncured allergic low molecular weight oligomers. Contact urticaria, allergic or irritant contact dermatitis caused by volatile compounds can also occur (62).

Acrylic resin cured by four different methods (conventional, microwave, injection molding and rapid heat cured resin) had a residual monomer content ranged from 0.045% to 0.103%, while auto-
polymerizing acrylic resin had a residual monomer content of 0.185%. It was reported that as much as 5% for self cured material may still be monomer after polymerization compared to 0.2% to 0.5% with heat cured type \(^{(63)}\).

The effect of eluates from heat activated, chemically activated and microwave activated denture base resins was examined on cell viability of primary cultures of human gingival fibroblasts in vitro. The cytotoxic effect was determined by cellular mitochondrial functions. The eluates from chemically activated resin disks were significantly more cytotoxic than the eluates from both microwave and heat activated resins \(^{(64)}\).

Denture wearing and patients associated factors such as changes in environmental conditions, trauma from ill-fitting denture, denture cleanliness and continuous denture wearing are considered the most important factors that predispose to oral Candidal infections. Oral Candidal growth and colonization are more pronounced in acrylic rather than metallic dentures. Wearing dentures both by day and night is associated with increased density and frequency of Candidal denture colonization and denture stomatitis \(^{(65)}\).

The importance of the condition of oral denture hygiene in regulating oral Candidal carriage have been investigated, denture plaque accumulating on the tissue surface of the denture as a result of poor denture hygiene, acts as a substrate that mediates *Candida albicans* colonization \(^{(65)}\).

Traumatic injuries produced by an ill-fitting denture may reduce
tissue resistance against infection and increase permeability of the epithelium to soluble Candidal antigens and toxins\(^{(65)}\).

**I.4. The Resident oral micro flora:-**

The oral flora is defined as “Those organisms that are present in a healthy mouth as a commensally and normal inhabitant without causing any disease”. The mouth supports the growth of a wide diversity of micro-organisms including bacteria, yeasts, mycoplasmas, viruses and (on occasions) even protozoa\(^{(66)}\).

**I.4.1. Bacterial genera found in the oral cavity:-**

*Marsh and martin classified the bacterial genera found in the oral cavity as follows:* \(^{(67)}\)

**Gram positive cocci include:**

*Abiotrophia, Enterococcus, Peptostreptococcus, streptococcus, staphylococcus and stomatococcus.*

**Gram positive rods include:**

*Actinomyces, bifidobacterium, Corynebacterium, Eubacterium, lactobacillus, propionibacterium, Pseudoramibacter and Rothia.* \(^{(67)}\)

**Gram negative cocci include:**

*Moraxella, Neisseria and veillonella.*
Gram-negative rods include:

Actinobacillus, Bacteriodes, Campylobacter, Cantonella, Capnocytophaga, Centipeda, Desulfovibrio, Desulfo bacter, Eikenella, Fusobacterium, Haemophilus, Johnsonii, Leptotrichia, Porphyromonas, Prevotella, Selenomonas, Simonsiella, Treponema and Wolinella
text (67).

McGhee et al pointed out that at birth the oral cavity is usually sterile but it may be contaminated with a several types of micro-organisms such as streptococci, staphylococci, coliform bacilli and gram positive rods. The source of these bacteria is the environment to which the child is gradually exposed during and after birth. The early oral micro flora after birth is mainly aerobic and facultative anaerobic (68).

The eruption of teeth, causes anaerobes as spirochetes, bacteroids especially beta melaninogenieus, fusobacterium, lactobacilli, actinomyces and some anaerobic vibrios to establish, streptococcus mutans and streptococcus sangius do not appear to become established until the eruption of teeth. However, the complete loss of the dentition causes a reversion of the micro flora to a predominately aerobic facultative type. Reduction in the number of streptococci and yeasts has been reported. The number of the yeasts was found to return to their pre-extraction levels after wearing dentures (69).

It was demonstrated that denture wearing encourages the growth of Candida species, staphylococci, streptococci, Neisseria and Diphtheroids (66).
I.4.2. Oral fungi:-

Stenderup reported that fungi other than yeasts cannot be considered as normal inhabitants and are rarely isolated from oral cavity of healthy individuals. Fungi are divided into perfect fungi, which reproduce sexually (i.e.: Fusion of two gametes) and imperfect fungi, which divide by a sexual reproduction. The perfect fungi are rarely isolated from the oral cavity but are occasionally found infecting patients with advanced AIDS. In contrast, the imperfect yeast is commonly found in the mouth. The largest proportion of the fungal micro flora is made up of Candida species, *Candida albicans* (C. albicans) is by far the most common species, but a large number of other yeasts have been isolated, including *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis* and *C. guilliermandi*, as well as *Rhodotorula* and *saccharomyces* species\(^{(70)}\).

*Candida albicans* could be isolated from the mouths of over 23% of normal patients having natural or artificial teeth. Furthermore, the prevalence of *Candida* species in the mouths of 140 infants at birth was 5.7%, which increased to 82% at age of 1 month and declined to 60% by 8 month of age. No correlation between the presence of maternal vaginal *Candida* and *Candida* in the mouths of infants was reported \(^{(71)}\).

I.5. *Candida albicans*:-

*Candida albicans* is part of normal flora and can be cultivated from the mouth of approximately 50% of adults \(^{(72)}\).
Candida albicans is unicellular eukaryotic microorganisms which reproduce by budding. It is a dimorphic fungus, which exists in both blastospore (yeast) and mycelial forms, it is in fact a trimorphic fungus because when it is put into certain specialized growth media (e.g: corn meal agar), small highly refractive spores called chlamydospore are formed. The transformation from a blastospore to a mycelium may occur. Both are found in infected tissue and on colonized surface, but the mycelial growth of elongated by hyphae invades the tissues. Thus it is usually accepted that Candida albicans in the mycelial phase is a parasite but, a saprophyte in the blastospore phase. Therefore, the isolation of hyphal structures is an indication of Candidal infection whereas; the more isolation Candida albicans is considered an unreliable proof of Candidal infection (67).

Candida albicans is considered the most common as well as the most pathogenic of all Candida species followed by C. tropicalis, C. stellalodia, C. parapsilosis, C. pseudotropicalis, C. krusie, C. guillermondii and C. glabrata (65).

Moreover, the organism can adapt to variable host environmental conditions through phenotypic variability (switch phenotype phenomenon) and development of drug resistance (73, 74).

Candida albicans and related species are highly successful opportunistic pathogens that reside in a benign state as commensally in the oral, digestive and vaginal cavities when the host is immunologically compromised or undergoes physiological changes, Candidal infection can ensue (75).
I.5.1. Intra-oral distribution of *Candida albicans*:

*C. albicans* was reported to be present in the vicinity of carious lesions,\(^{(76)}\) plaque coated surfaces of teeth,\(^{(77)}\) sub gingival flora, gingival tissues of advanced periodontal abscesses, and in advanced periodontitis of AIDS patients\(^{(78)}\).

In healthy dentate individuals, *C. albicans* was found not to be uniformly distributed throughout the mouth, the dorsum of the tongue particularly its posterior half area near circumvallate papillae as the sole oral reservoir of the fungus. It was claimed that, the tongue papillae provide a large surface area for adherence of microorganisms and shelter them from removal during eating and swallowing\(^{(77, 79)}\).

The isolation of Candida is raised by the presence of intra oral devices such as dentures or orthodontic appliance, particularly on the fitting surface of the upper acrylic dentures. Plaque has been also shown to contain Candida species\(^{(66, 79)}\).

I.5.2. Oral Candidal infection:-

Although *C. albicans* are usually encountered as oral commensals, they are found to be responsible for a multiple oral infections.\(^{(65)}\) These opportunistic infections occur in cases of local or systemic derangements in host resistance\(^{(73)}\).

Holmstrop and Axell \(^{(80)}\) have classified, oral Candidal infections, clinically, into the following types:-
1- Acute types (Pseudomembranes and erythematous).

2- Chronic types (pseudomembranes, erythematous, plaque like and nodular).

3- Candida- associated lesions (Angular cheilitis, glossitis and denture stomatitis)

Samaranayake, \(^{(74)}\) classified oral Candidosis as:

- Primary oral Candidosis in which localized Candidal infections are present only in the oral and perioral tissues.

- Secondary oral Candidosis which refers to Candidal infections that are manifested in a generalized manner both in the oral cavity and in the other mucous and cutaneous surface (systemic mucocutaneous Candidal infection).

The oral lesions of both the primary or secondary oral Candidosis may appear as pseudomembranous, erythematous (atrophic) or hyperplastic.

Bagg et al, \(^{(79)}\) classified oral Candidosis into:

- Acute pseudomembranous Candidosis (oral thrush).

- Acute erythematous Candidosis (atrophic Candidosis).

- Chronic erythematous Candida (denture stomatitis).

- Chronic plaque-like and nodular (Candidal leukoplakia).
Acute or chronic angular cheilitis.

Pseudomembranous Candidosis (thrush) is an acute infection, which may persist intermittently for many months or even years in immune-compromised patients, or those under corticosteroid therapy, neonates and patients with terminal illness. It is characterized by discrete white lesions that may be formed on the buccal mucosa, tongue, hard palate and throat\(^{(66)}\).

On the other hand, erythematous (atrophic) Candidosis may arise as a consequence of persistent acute Pseudomembranous Candidosis when the Pseudomembranes are shed or may develop de novo. Erythematous Candidosis of the palate is frequently observed in elderly and full denture wearers in the form of erythematous lesions of varying severity confined to tissues underlying the denture surfaces. Hyperplastic Candidosis are present as chronic discrete raised areas that vary from small palpable translucent white area to large dense opaque plaque-like lesions\(^{(81)}\).

I.5.3. Factors predisposing to oral Candidal infection:

Multiple factors predispose for oral Candidal growth and colonization. Local and systemic factors so frequently permit Candida to cause disease that it is extremely rare to find a case of oral Candidosis in which one or more of these factors cannot be identified\(^{(65,82)}\).

I.5.3.1. Local predisposing factors:

Local factors include: trauma and malocclusion associated with denture wearing, topical application of antibiotic or steroids which may
result in marked change in oral microbial flora, excessive use of antibacterial mouth rinse and heavy smoking.

### I.5.3.2. Systemic predisposing factors:

Systemic factors include:

1) Salivary factors: xerostomia, Sjogren’s syndrome, radiotherapy and cytotoxic therapy.

2) Diet factor: as high carbohydrates diet.

3) Infection factors: any systemic long standing infections, HIV infection.

4) Physiological infancy and old age.

5) Hormonal factors: diabetes, hypothyroidism, hyper-parathyroidism and hypoadrenocortical active.

6) Nutritional factors: hypovitaminosis, iron-deficiency and malnutrition.

### I.6. Denture stomatitis:

(Chronic atrophic Candidosis or denture sore mouth)

Denture stomatitis is the most commonly encountered intra-oral pathological condition among denture wearers\(^{(83)}\).

Denture stomatitis refers to inflammatory changes of the denture bearing mucosa, which may appear erythematous, spongy and sometimes may show papillary hyperplasia\(^{,(84,85)}\).
Bergendal and Isacsson, observed that denture stomatitis more often affects the maxillary than the mandibular denture bearing mucosa. Their results demonstrated that although the condition may involve the entire upper denture bearing area, it is more often confined to the mucosa of the palatal vault (86).

Regarding the possible etiologic factors, many investigators (87-90) believe that the cause of denture stomatitis is multifactorial, being related to Candidal infection, denture trauma, denture cleanliness and allergic reaction to denture base materials. However, others believe that no primary etiological factor exists (Bergendal (91); Arendorf and Walker (84)).

Although bacteria and / or other yeasts may act as pathogens in denture stomatitis, *C. albicans* has been implicated as the principal factor in the initiation, aggravation and maintenance of denture stomatitis (Renner at al., (92); Arendorf and Walker (84).

Many investigators (87, 93-95) were able to demonstrate a positive correlation between the agar colony location, quantity of *C. albicans*, and the clinical location and severity of inflammation on the patient’s palate.

The response of denture stomatitis to antifungal therapy provides evidence for the primary role of *Candida albicans* in inducing denture stomatitis (Quinn,) (96). However, controversy has arisen to whether *C.albicans* is superimposed upon traumatized tissues from ill fitting denture base (87), or whether the inflammation is caused directly by *C.albicans* (95).

Kamalkshi et al. , used peridex rinse for the treatment of denture
stomatitis. The results showed that pre-treatment and post-treatment localization of *C. albicans* on the denture surface, were strikingly similar\(^{(93)}\).

**I.7. Pathogenicity of *Candida albicans* in denture stomatitis:**

The pathogenicity of *C. albicans* has been thought to depend on the number of the organism. Santarpia\(^{(94)}\), showed that the more the number of yeasts adhering to the denture, the greater the potential for an increased inflammatory reaction. The mechanisms by which *C. albicans* are assumed to cause denture stomatitis can be grouped as follows:

**I.7.1. Tissue Invasion:**

There is an agreement that after the adherence and colonization of *C. albicans* on the fitting surface of acrylic dentures, it invades the epithelial cells by hydrolytic enzymes, which is followed by an acute inflammatory response\(^{(88, 97-99)}\).

Kamalakshi et al Suggested that the inflammatory response on the palatal mucosa, is influenced by direct yeast invasion of the mucosa and by recurring infection of the palate by *C. albicans* adhering and growing on the denture\(^{(93)}\).

**I.7.2. Allergic or immune response (delayed hypersensitivity reaction):**

*C. albicans* synthesizes specific antigens on the surface of their cell walls. Once the Candida cell adheres to the mucosal surface, cell wall
components are released and penetrate the epithelium to start the cycle of inflammation\(^{100}\).

Impaired immune- response against \textit{C.albicans} has been reported in patients with Candida-induced denture stomatitis. However, after antimycotic treatment, immunity was restored, indicating that the suppression of cellular immunity against \textit{C.albicans} is most unlikely to be the direct cause for denture stomatitis\(^{99}\).

\textbf{I.7.3.Intra-Oral adhesion of \textit{C.albicans}}:

The ability of \textit{C.albicans} to adhere to epithelial cells or solid surfaces such as acrylic resins or denture lining materials, has been thought to be the initial step in the successful colonization, subsequent plaque formation and development of pathogenesis (Nikawa et al.,)\(^{101}\).

\textbf{I.7.4.Production of extra-cellular metabolic subustances}:

During growth and metabolism of \textit{C.albicans}, organic acids are produced, which account for the low pH between the fitting surface of the denture and the palatal mucosa. These organic acids may either have a direct cytotoxic effect on the mucosa, or the acidic pH may activate \textit{C.albicans} proteases and phospholipases causing mucosal inflammation\(^{102}\).

\textbf{I.8.Intra-oral adhesion of \textit{Candida albicans}}:

Adhesion of \textit{C.albicans} to oral mucosa and denture surface is
probably an important initial step in the pathogenesis of oral Candidal infections (Gibbons and Van Houte, \(^{(103)}\); Olsen, \(^{(104)}\)).

Many reports \(^{(105-110)}\) have focused on clarification of the nature of adherence of *C. albicans* to denture bases and factors affecting it.

The effect of saliva on the adherence of *C. albicans* has been widely investigated by many researchers \(^{(104, 106, 111-113)}\). The results showed great controversy, suggesting that the role of saliva in adhesion of *C. albicans* adhesion is rather complex.

Jendresen and Glantz \(^{(114)}\) demonstrated that the adhesive properties of any artificial surface are modified in the oral environment, owing to the acquired salivary pellicle that rapidly forms on it. They concluded that different surfaces are quickly to the same state by the absorption of a surface salivary film. The salivary pellicle, coating mucosal epithelial cells and denture surfaces, has been shown to enhance Candidal adhesion and colonization \(^{(106, 112)}\).

Secretory immunoglobulin A, present in saliva, was proved to inhibit binding of *C. albicans* to epithelial cells \(^{(104)}\). Also, pre-treatment of denture acrylic specimens with unstimulated mixed saliva for 30 minutes resulted in reduced adherence for all Candida strains \(^{(111)}\).

Vasilas et al. \(^{(113)}\) observed that salivary components enhanced *C. albicans* adherence to saliva coated denture acrylic specimens, suggesting that acquired salivary pellicle may play an important part in the
colonization of the acrylic denture by *C. albicans*.

Different degrees of cell surface hydrophobicity of Candida species have been correlated with the ability of the yeasts to adhere to denture base materials \(^{(108)}\). In an attempt to explain the nature of the role of saliva in Candidal adhesion, it was suggested that this could be attributed to changes in relative hydrophobic properties \(^{(115)}\).

Binding of *C. albicans* to acquired denture pellicle has been reported to be mediated by specific salivary or serum components, which may provide receptor sites for specific adherence of the microorganism \(^{(110, 116)}\).

Denture pellicle promoted *C.albicans* colonization and hyphal invasion of denture base materials. The nature of salivary proteins bound to denture base materials by pellicle, may play an important role in *C.albicans* adherence, than the surface properties of the materials \(^{(110)}\).

Factors other than hydrophobic interaction, such as specific interaction have also been suggested to be greatly involved in the adherence of *C.albicans* to saliva coated denture base materials \(^{(110)}\).

In vitro studies on the adhesion of *C.albicans* to denture acrylic resin, demonstrated that pre-coating the samples with human serum, enhanced adhesion of yeasts cells \(^{(111)}\). This Finding was supported by other study who reported that serum transudate produced by inflamed palatal tissue, as a result of prosthetic trauma, may be incorporated in the adsorbed denture pellicle and enhances Candidal adhesion. Thus, creating disease
promoting pellicles that permit microbial colonization on the denture surface\textsuperscript{(107)}.

The growth and development of \textit{C.albicans} micro colonies are determined by the mechanical features of the denture fitting surface. Heat cured acrylic denture surface, being relatively smooth, offers little mechanical retention to denture plaque. Conversely, the surface of denture base materials which have been in service for some time, has been observed to be porous, comprising a series of depressions which make adequate denture hygiene difficult and thus, is more favorable for \textit{C.albicans} colonization\textsuperscript{(105)}.

Growth of \textit{C.albicans} detected on the denture surface, is associated with plaque accumulated as a result of poor denture hygiene, rather than by penetration of surface defects and irregularities\textsuperscript{(117)}.

The surface free energy of the denture material may also influence Candidal adhesion\textsuperscript{(118)}.

Exogenous and Endogenous carbon sources, may affect the oral carriage of \textit{C.albicans} cells by modifying their adhesive properties. The addition of glucose to nutrient –depleted saliva produced an exceptional growth of \textit{C.albicans}, despite the presence of a nutrient competing bacterial salivary flora\textsuperscript{(109)}.

Budtz-jorgensen,\textsuperscript{(88)} suggested that high carbohydrate intake may predispose to Candida-induced denture stomatitis. This was supported by Samaranayake and MacFarlane,\textsuperscript{(119)} who showed that pre-incubation with
sucrose, greatly increased the adherence of *C. albicans* to acrylic strips. They suggested that an extracellular metabolic product of the organism could be responsible for the enhanced adhesion associated with sucrose. They added that, the soft carbohydrate rich diet consumed frequently by denture wearers, could induce yeast to colonize and adhere more tenaciously to denture surfaces and thus, could play an important role in the pathogenesis of denture stomatitis.

In the absence of nutrient sources of carbon, denture base materials failed to support the growth of *C. albicans*. Some organisms can attack denture base materials, freeing carbon for their use as an essential nutrient

I.9. Laboratory diagnosis of denture stomatitis:

**Isolation and identification of Candida albicans:**

Olsen and Stenderup suggested two techniques for fungal identification, which are smears and swabs.

**I.9.1. Smears:**

The detection of yeast in a clinical specimen should start with direct microscopic examination of unstrained smears from the lesion.

Smears are taken from the infected areas intra orally and from the fitting surface of the denture, with wooden spatulas and then the material is pressed between the two glass microscopic slides and then fixed
immediately with ether alcohol 1:1 or with spray fix.

After fixation, one slide is stained with gram and the other with periodic acid Schiff (PAS). Yeast cells appear dark blue after gram staining and red in (PAS) preparations.

The presence of large amounts of blastospores and hyphae is indicative of Candidal infection, although hyphae may be more dominant than blastospores in smears from clinical lesions. Also, another indication of Candida-infected lesion is the presence of large accumulations of inflammatory cells in direct smears\(^{(121)}\).

**I.9.2. Swabs:**

The area to be sampled was rubbed vigorously with sterile swab for 20 seconds, the authors stressed on the importance of firm swabbing with a moistened swab as organisms may be deeply seated. Then swabs were seeded on the cultures plates containing either sabourad’s agar (25\(^0\)C or room temperature) or blood agar (35\(^0\)C) or pagno- Levin medium (35\(^0\)C) or littmann’s substrate (25\(^0\)C).

Sabourad’s dextrose agar may not always be the best for distinguishing between multiple yeast species while pagano-levin medium or littmann’s substrate enable distinction of yeasts on the basis of difference on colony color.

The most common yeasts form colonies within 1-3 days of incubation. Identification of *C. albicans* could be obtained by
morphological examination of the resulted colony (121).

I.9.3. Imprint culture technique:

This technique was developed by Arendorf and Walker (1980), they used a sterile foam pad (2 x 2 cm), dipped in peptone water and then placed on a restricted area of the oral mucosa for 30 sec. Thereafter the pad is placed directly on pagano-levin or sabourad agar, and the growth of Candida is quantified. This technique may be useful for assessing yeast growth in different areas of the oral mucosa and the denture (122).

I.9.4. Oral rinse technique:-

In this technique, the patients were instructed to rinse the mouth with 10 ml of sterile phosphate buffered saline for 60 seconds. The rinse was then expectorated into a universal container and immediately transported to the laboratory for concentration by centrifugation, then cultured on sabouraud’s dextrose agar plates which where incubated at 37°C for 48 hours (123).

Lamey and Samaranayke recommended oral rinse or imprint culture for differentiating between commensal yeast carriage and clinical Candidal infection (124).

I.9.5. Germ tube test:-

Many methods for identification and sensitivity testing of yeasts were introduced; one of them is the germ tube test, which is a rapid, simple
and very valuable test for the identification of *Candida albicans*.

A germ tube is a filamentous extension from a yeast cell that is about one half the width and three to four times the length of the cell. Germ tubes appear when *Candida albicans* isolated are added to 0.5 to 1.0 ml of serum (human or, sheep or horse serum) so as to make a cloudy suspension, then the mixture is incubated at 37°C for 2 to 3 hours, and then a drop of the suspension is examined microscopically for germ tubes. A filamentous outgrowth from a yeast cell is seen with no constriction present at the base, it is only the yeast *Candida albicans* that produce germ tubes. Early pseudohyphae of *Candida tropicalis* may be confused but characteristically show a point of constriction adjacent to the mother cell. Only *Candida albicans* produce germ tubes although *Candida stellatoidea* may also produce germ tubes, but it is usually regarded as a variant of *Candida albicans* (125).

**Chlamydospore formation:-**

The ability of the genus Candida to induce chlamydospore formation is used as an important identification criterion. In the majority of *Candida albicans* isolated (> 90%), the characteristic chlamydospores are produced when the isolated are cultured on corn meal or rice-tween 80 agar. This characteristic is a consistent in *Candida albicans* as the formation of germ tubes (126).
I.9.6. Morphological investigation by Scanning electron microscope:

In this technique, small agar blocks (5x2x5mm) bearing fungal structures are fixed using the method described by Lee et al. (2003), after fixation, samples and dried and coated with gold palladium and examined using scanning electron microscope.
The Aim of this study was to evaluate and compare the effect of conventional heat-cured and microwave-cured acrylic denture resin on Candidal growth.
II-MATERIALS AND METHODS

II.1.Patients Selection:

Seven Completely edentulous co-operative male patients were selected from the out-patient clinic, Prosthodontic Department, Faculty of Dentistry, Ain-Shams University.

II.1.1Criteria for patients selection:

- Patients age ranged between 40 and 60 years.
- All patients were non-smokers and had no previous denture experience.
- All patients were apparently in good general health, free from systemic diseases that may affect the oral condition e.g.: diabetes mellitus, anemia and immune-deficiency states as indicated by the medical history.
- Patients had well formed residual ridges free from severe bilateral undercuts, bony specules or sharp ridges.
- Patients had healthy mucosal coverage free from any signs of inflammation, ulceration or hyperplasia.
- Patients had normal ridge relationship (Angle’s class I) and adequate interarch space.
Patients with excessive salivation or thick ropy saliva were excluded. Those having dry mouth were not also considered.

- All patients had no Temporo-mandibular joint disorders.

- All patients had no Para-functional habits as bruxism, clenching or tongue thrusting.

Patients were informed about the nature of this research work. Their approvals were obtained. Only those who showed co-operation and adherence to treatment and recall appointments were included.

Patients were asked about their past and present medical condition. They were asked to perform laboratory investigations that included complete blood picture, glucose tolerance and alkaline phosphatase test to ensure the absence of any systemic diseases that might contribute to bone resorption.

II.2. Patient’s examination:

II.2.1. Examination of the temporomandibular joints:

Examination of the temporomandibular joints was carried out during opening, closing and lateral movements to
exclude any temporomandibular joint disorders or tenderness of the masticatory muscles.

II.2.2. Intra oral examination:

Full clinical examination was made for the residual ridges to fulfill the predetermined criteria.

-Mucosa of the edentulous area was examined both visually and digitally to detect any signs of inflammation, pathology or tissue flabbiness.

Maxillary and mandibular ridges were examined for the presence of any bony undercuts, sharp ridge, tori or any abnormality.

II.2.3. Ridge relationship evaluation:

Upper and lower alginate impressions were made for the selected patients and poured into dental stone to obtain diagnostic casts. A tentative centric jaw relation was made and casts were mounted on fixed condylar path articulator to evaluate the interocclusal distance and ridge relationship. Only patients with adequate interocclusal distance and normal ridge relation were included in the study.
II.3. Grouping of Patients:

The selected patients were re-habilitated with heat-cured mucosa supported complete denture. Dentures were constructed following the conventional technique and monoplane concept of occlusion.

At the time of denture insertion and one month after performing the post insertion adjustment, salivary samples were collected to evaluate the Candidal growth following the oral rinse technique.

One month resting period was allowed for the oral microbial flora to return to normal by taking off the dentures from the patients, after that, the Dentures were rebased using microwave cured acrylic resin.

Before the insertion of the rebased dentures and one month following the post insertion adjustment, salivary samples were collected to evaluate Candidal growth using the oral rinse technique.

II.4. Denture Construction:

For all the patients, upper and lower complete dentures were constructed as follows:
- Preliminary alginate* impressions were made for the upper and lower arches in properly selected and adjusted aluminum stock trays as shown in fig (1).

- Impressions were poured in dental stone to produce study casts, upon which selectively relieved acrylic resin special trays were constructed 2 mm short of the muco-buccal fold and small holes were done to allow the escape of the impression material.

- Border molding was done using medium consistency rubber base material**.

- Secondary impressions were made in a border molded special acrylic trays using also medium consistency rubber base material under light finger pressure as shown in fig(2).

- The impressions were poured in dental stone to have master casts on which occlusion blocks were made for recording the jaw relation.

- The casts were mounted on a fixed condylar path articulator at the predetermined vertical dimension of occlusion following the inter occlusal wax technique using the check bite technique and was locked in centric position.

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** Impregum F, Polyether impression material, hydrophilic, medium consistency ISO 4823 type 2, made in Germany by 3M ESPE AG, D-82229 seefeld.
Fig. (1) A, B and C; A, Primary upper alginate impression; B, Primary lower alginate impression and C, Primary upper and lower alginate impression.
Materials and Methods

Fig. (2)  A, B and C; A, Secondary upper rubber base impression; B, Secondary lower rubber base impression and C, Secondary upper and lower rubber base impression.
- Non-anatomic cross linked acrylic teeth were arranged following the guide lines of the monoplane concept of occlusion (127).

- The upper and lower anterior teeth arranged without vertical overlap.
- The lower occlusal plane was adjusted parallel to the mean foundation area.
- The height of the occlusal plane was made at the junction of the upper and middle third of the retromolar pad.
- Lower posterior teeth were positioned in a horizontal plane antero posteriorly and mediolaterally.
- The upper second molar was either omitted or arranged 2 mm. out of occlusion.

- The waxed up dentures were tried in the patient’s mouth, to check extension, retention, stability, even bearing on both sides and correct occlusion as shown in fig (3).

- Denture Processing was carried out using heat cured acrylic resin following long curing cycle (74 C° for Six hours).

- After deflasking, laboratory remounting was carried out to refine occlusion and correct the processing occlusal errors. Dentures were decasted, finished and polished.
Fig. (3) Try-in stage in denture construction.

-Dentures were stored in tap water for 24 hours before delivery.

-The finished dentures were delivered to the patient after performing the needed occlusal adjustment as shown in fig (4). Patients were asked to contact if any pain is experienced, one and two weeks later to perform any needed post insertion adjustments.

-Before dismissing the patients salivary samples were taken to estimate the Candidal count.
-Patients were appointed one month after performing the needed post-insertion adjustments for collection of the samples, evaluation of the prosthesis as well as the condition of the denture bearing mucosa.

Fig. (4) Finished and delivered conventional heat-cured upper and lower complete denture

II.5. Patient instructions:

At the time of denture insertion, patients were instructed to perform oral and denture hygiene as follow:

- The prosthesis should be left out of the mouth for approximately 6 to 8 hours every 24 hours period and placed in a container containing tab water.
Materials and Methods

- Brushing the prosthesis after each meal by soft denture brush preferably over a basin partially filled with water to prevent accidental drop and breakage.

- The use of chlorohexidine mouth wash with the prosthesis out of oral cavity, as the solution normally stains the artificial teeth and denture base material.

- Tooth paste and solutions containing phenol should be avoided to prevent abrasion and crazing of the denture.

- Also hot water should be avoided as it may cause warpage of acrylic resin.

Then the patients were instructed to remove their dentures for one month in order to allow the Candida to reach its baseline. Samples were repeated to insure that Candida reach baseline, and then dentures were rebased using microwave-cured acrylic resin.

II.6. Rebasing:

Dentures were rebased using microwave cured acrylic denture base resin as follow:

- The fitting Surfaces and Flanges were reduced 1-2mm to give adequate room for impression materials and remove any area of undercuts.
- A new border tracing was done using Medium consistency Rubber base material** and the patient was asked to close in centric.

- A stone cast was poured.

- The dentures attached to the poured cast were mounted on upper member of the articulator, whereas an occlusal index was established on lower member of the articulator as shown in fig (5).

- Rebasing was done by replacement of all the old denture base material by a microwave cured acrylic denture base without changing the arrangement of the teeth.

- Acrylic resin teeth were cut from denture base with a bur as shown in fig (6) and then seated in their indentations in occlusal index as shown in fig (7).

- Waxing up was done.

- Flasking was done using microwave flask as shown in fig (8). Acrylic resin powder and liquid were mixed so as to assure proper wetting of all powder particles, the Mixing time allowed was 15-30 seconds and the working time was 10 ±4 minutes. After that packing was done in **Impregum F, Polyether impression material, hydrophilic, medium consistency ISO 4823 type 2, made in Germany by 3M ESPE AG, D-82229 seefeld.
the microwave flask, where packing was identified when resin was in the dough stage.

- Curing of the microwave cured acrylic resin was done by inserting the microwave flask in the microwave device for 5-6 minutes.

- After deflasking, laboratory remounting was carried out to refine occlusion and correct the processing occlusal errors. Dentures were decasted, finished and polished.

- At the time of denture insertion of the microwave-cured denture and one month after performing the needed post insertion adjustments, salivary samples were collected to evaluate Candidal growth using the oral rinse technique.
Fig. (5, A) A, upper complete Denture mounted on upper member of the articulator, B, C and D, occlusal index being established on lower member of the articulator.
Fig. (6) Acrylic resin teeth cut from denture base using a bur.

Fig. (7) Acrylic resin teeth after being cut from denture base was seated in their indentations in occlusal index on lower member of the articulator.
Fig. (8, A) and B, microwave flask; A, microwave flask assembly and B, microwave flask parts.
II.7. Collecting the Samples:

Oral rinse technique was used which suggested by Stendrup\(^{(70)}\) and adopted by Williams et al\(^{(123)}\).

The patients were instructed to rinse their mouths with 10mL of sterile phosphate buffered saline for 60 seconds. The rinse was then expectorated into a universal container and immediately transported to the laboratory for concentration by centrifugation as shown in fig (9,A), then cultured on sabouraud’s dextrose agar plates which were incubated at 37 C° for 48 hours.

Candidal colonies appeared to be white to creamy in color, smooth and glistening as shown in fig (9, B). If no colonies were visible, a negative result was recorded as shown in fig (9, C).

The colony forming units of the organism were quantified according to the scale developed by Olsen\(^{(104)}\) and adopted by Bergendal et al.\(^{(128)}\) as follows:

No colonies= 0 ; 1-100 colonies= 1 ; more than 100 colonies= 2 ; confluent growth= 3.
Fig. (9, A), (B) and (C); A, representative samples of oral rinse of two patients using the oral rinse technique; B, Negative growth of *Candida albicans* culture from sample four shown in fig. 9,A (arrowed) grown on sabouraud’s dextrose agar medium for 48H at 37°C; and C, positive growth of *Candida albicans* culture from sample three shown in fig. 9,A (arrowed) grown on sabouraud’s dextrose agar medium for 48H at 37°C.
II.8. Light microscopic examination:

Smears from the colonies were prepared, stained with gram stain and examined by oil-immersion lens of the microscope for the presence of budding oval gram positive yeast cells and pseudohyphae of Candida. The colonies were sub cultured on sabouraud’s dextrose agar slopes with chloramphenicol to be subjected to the germ tube test for identification of *Candida albicans as shown in figures(10) and (11).*

Fig. (10), Light microscopy of *Candida albicans* grown on sabouraud’s dextrose agar medium stained by gram stain (+ve) showing budding stage (arrowed). Bar scale: 2.5µm.
**Materials and Methods**

Fig. (11) Light microscopy of *Candida albicans* grown on sabouraud’s dextrose agar medium stained by gram stain (+ve) showing various fungal structures [hyphae (arrowed) and germ tube (GT)]. Bar scale: 2.5µm.

**II.8.1. Germ tube test:**

Candida cells were picked by touching a single pure colony lightly with a sterile loop. The cells were suspended in 0.3-0.5mL human serum in Wassermann tube. The serum culture was incubated at 37 C for 2.5-3 hours. Using the Loop, One drop of the serum culture was placed on a clean slide, covered with a glass cover followed by microscopic examination developed by Sandven. Germ tubes appeared as cylindrical narrow filaments with no constriction at the point from the mother cells. The presence of germ tubes is characteristic for *Candida albicans as shown in fig (12).*
Fig. (12), Light microscopy of *Candida albicans* grown on human serum for 2-3 h. at 37°C showing Conidia(C), Germ tube (GT) and aggregates of spores in clusters (arrowed). Bar scale: 2.5µm.

**II.9. Morphological investigation by scanning electron microscope:**

For scanning electron microscopy (SEM), small agar blocks (5×2×5 mm.) bearing fungal structures were fixed using the method described by Lee et al. (2003). After fixation, samples were dried with a Blzers CPD 020 critical point
drier. They were coated twice with gold palladium using a Nanotech Semprep II sputter coater and examined using a Hitachi S 4700 scanning electron microscope at the Regional center for fungi, Azhar University.
III-RESULTS

The results of this study were represented in tables (1-4) and figures (13-16). Testing for significance between the Candidal colony forming units within each denture base before denture insertion and one month after insertion was carried out using Fisher’s exact test.

Fisher’s exact test was also used to compare between the prevalence of the colony forming units in the two studied denture base (conventional heat-cured and microwave-cured acrylic denture resin) before and one month after denture insertion. Probability level (P<0.05) was considered statistically significant.

III.1. The effect of conventional heat-cured acrylic denture resin on Candidal growth:

Tables (1) and figure (13) represent the prevalence of Candidal forming units in group (I) before and one month after denture insertion and their level of significance.

Before denture insertion, three of the studied cases (42.85%) showed zero score (no growth). While score 1 (1<CFU<100) was detected in the other four studied cases (57.14%).
Results

One month after denture insertion of the conventional heat-cured acrylic denture base resin, the prevalence of score zero (no growth) was detected in only one case (14.28%), score 1(1<CFU<100) was detected in three studied cases (42.85%) and score 2(CFU>100) was detected in the other three studied cases (42.85%).

Comparing the prevalence of colony forming units (CFU) before denture insertion and one month after insertion, the data obtained by applying Fisher’s exact test revealed insignificant difference (P>0.05) as shown in table (1).

Table (1): The prevalence of colony forming unit (CFU) and fisher’s exact test in heat cured acrylic denture base during the follow up cases

<table>
<thead>
<tr>
<th>CFU</th>
<th>Zero time</th>
<th>One month</th>
<th>Fishers</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score</td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Group I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>42.85%</td>
<td>1</td>
<td>14.28%</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>57.14%</td>
<td>3</td>
<td>42.85%</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0%</td>
<td>3</td>
<td>42.85%</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

No growth=score zero 1<CFU<100=score 1
CFU>100=score 2        confluent growth=score 3
III.2. The effect of microwave-cured acrylic denture resin on Candidal growth:

Table (2) and figure (14) represent the prevalence of Candidal forming units in group (II) before and one month after denture insertion and their level of significance.

Before denture insertion, one of the studied cases (14.28%) showed zero score (no growth) while score 1 (1<CFU<100) was detected in the other six studied cases (85.714%).
One month after denture insertion of the microwave-cured acrylic denture base resin, the prevalence of score zero (no growth) was detected in two cases (28.57%), score 1 (1<CFU<100) was detected in four studied cases (57.14%) and score 2 (CFU>100) was detected in only one case (14.28%).

Comparing the prevalence of colony forming units before denture insertion and one month after insertion, the data obtained by applying Fisher’s exact test revealed insignificant difference (P>0.05) as shown in table (2).

Table (2): The prevalence of colony forming unit (CFU) and fisher’s exact test in microwave-cured acrylic denture base during the follow up cases

<table>
<thead>
<tr>
<th>CFU Score</th>
<th>Zero time</th>
<th>One month</th>
<th>Fishers (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Group II</td>
<td>0</td>
<td>1</td>
<td>14.28%</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6</td>
<td>85.714%</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

No growth=score zero  1<CFU<100=score 1  
CFU>100=score 2  confluent growth=score 3

Group (II): Microwave-cured acrylic denture.
Figure (14): The prevalence of colony forming unit (CFU) scores within group (II) before and after one month of the denture insertion.

III.3. Comparison between conventional heat-cured and microwave-cured acrylic denture resin on Candidal growth:

To compare between the effect of denture base material (heat-cured and microwave-cured) on the prevalence of colony forming units, Fisher’s exact test was carried out and the results are shown in figure (15-16) and table (3-4).

Table (3) and figure (15) represent the prevalence of colony forming units before insertion of conventional heat-cured and microwave-cured acrylic denture base.
The data obtained from the table (3) show that zero score (no growth) was detected in three cases (42.85%) and one case (14.28%) before using the conventional heat-cured and microwave-cured acrylic denture base resin respectively.

While the Colony forming units score 1 (1<CFU<100) was detected in four cases (57.14%) and six cases (85.714%) before insertion of heat-cured and microwave-cured acrylic denture base resin respectively.

Statistical analysis of the data revealed insignificant difference between colony forming units (P>0.05).

Table (3): Comparison between the effect of conventional heat-cured and microwave-cured acrylic denture on candidal growth before denture insertion.

<table>
<thead>
<tr>
<th>CFU Score</th>
<th>Group (I) before denture insertion</th>
<th>Group (II) before denture insertion</th>
<th>Fishers</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>42.85%</td>
<td>1</td>
<td>14.28%</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>57.14%</td>
<td>6</td>
<td>85.71%</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

No growth=score zero   1<CFU<100=score 1

CFU>100=score 2   confluent growth=score 3


Group (II): Microwave-cured acrylic denture
Figure (15): The prevalence of Colony forming units (CFU) in conventional heat-cured and microwave-cured acrylic denture base resin before denture insertion.

Table (4) and figure (16) represent the prevalence of colony forming units after one month insertion of conventional heat-cured and microwave-cured acrylic denture base. The morphological features of *Candida albicans* sampled from representative patients i.e.: no.5 and no. 7 as described previously in the materials and method, revealed with patient no.5 the formation of different forms of budding after 1 month wearing the conventional heat cured complete denture as shown in fig. (17). While after one month of wearing the microwave cured complete denture the scanning electron micrographs revealed in addition to the previously seen budding forms with the conventional heat cured complete
denture, the formation of germ tubes (GT) and pseudohyphae surrounded by mucilaginous layer as shown in fig. (18). However, with samples obtained from patient no. 7 after 1 month wearing the conventional heat cured complete denture the scanning electron microscope revealed, different developmental stages of *Candida albicans* that is Conidia(c), budding forms and pseudohyphae formation as shown in fig. (19), while after 1 month of wearing microwave cured complete denture the scanning electron microscope revealed only budding formation (without formation of germ tubes or pseudohyphae) as shown in fig. (20).
Results

Fig. (17, A)

Fig. (17, B)

Fig. (17, C)
Fig. (17) A, B, C, D and E, Scanning electron microscopy photography of *Candida albicans* sampled from patient no. 5 one month after wearing conventional heat-cured complete denture; A, general view of *Candida albicans*; B, enlarged portion of insert shown in Fig. (17, A); C, mono-form budding (arrowed); D, bi-polar budding (arrowed) and E, tri-polar budding (arrowed). Bar scale; A, 10µm; C, D and E, 2µm.
Fig. (18) A and B, scanning electron microscopy photography of *Candida albicans* samples from patient no. 5 after one month wearing microwave-cured complete denture; A, showing budding stage of different forms (arrowed) and germ tube (GT); B, mucilaginous material surrounding the Pseudohyphae (arrowed). Bar scale; A, 5µm and B, 2µm.
Fig. (19, A) and (B)

Fig. (19) A and B, Scanning electron microscopy photography sampled from patient no. 7 after one month wearing conventional heat-cured complete denture showing developmental stages of *Candida albicans*; A, ungerminated Conidia (C) and budding of *Candida albicans* (arrowed); B, Pseudohyphae (arrowed). Bar scale A and B, 5µm.
Fig. (20) Scanning electron microscopy photography sampled from patient no. 7 after one month wearing microwave-cured complete denture showing different budding stages (arrows). Bar scale 2µm.

The data obtained from the table (4) showed that zero score (no growth) was detected in only one case (14.28%) and two cases (28.57%) after one month using the conventional heat-cured and microwave-cured acrylic denture base resin respectively.

The colony forming units score 1 (1<CFU<100) was detected in three cases (42.85%) and four cases (57.14%)
Results

after one month insertion of heat-cured and microwave-cured acrylic denture base resin respectively.

While the colony forming units score 2 (CFU>100) was detected in three cases (42.85%) and only one case (14.28%) after one month insertion of heat-cured and microwave-cured acrylic denture base resin respectively.

Statistical analysis of the data revealed insignificant difference between colony forming units (P>0.05).

Table (4): Comparison between the effect of conventional heat-cured and microwave-cured acrylic denture on Candidal growth after one month interval.

<table>
<thead>
<tr>
<th>CFU Score</th>
<th>Group (I) after 1 month</th>
<th>Group (II) after 1 month</th>
<th>Fishers</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>14.28%</td>
<td>2</td>
<td>28.57%</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>42.85%</td>
<td>4</td>
<td>57.14%</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>42.85%</td>
<td>1</td>
<td>14.28%</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

No growth=score zero 1<CFU<100=score 1 CFU>100=score 2 confluent growth=score 3

<table>
<thead>
<tr>
<th>CFU Score</th>
<th>CFU Score</th>
<th>CFU Score</th>
<th>CFU Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

Figure (16): The prevalence of Colony forming units (CFU) in conventional heat-curd and microwave-cured acrylic denture base resin one month after denture insertion.
Discussion

Patients selection was mainly directed towards elimination of the possible factors that could affect Candida albicans count. Female patients did not participate in this study, to avoid the possible associated menopausal and post-menopausal changes on the supporting structures\(^\text{(95, 129)}\). Also, to avoid variations in the salivary flow between males and females, and to overcome the high oral yeast count which has been reported among females\(^\text{(130)}\).

To eliminate the effect of age changes on the oral microbial flora balance, the age of all subjects selected ranged between 40-60 years\(^\text{(131)}\).

Some systemic diseases such as, diabetes mellitus, anaemia and immunodeficiency states have been reported to cause imbalance of the oral microbial flora and to adversely affect tissue tolerance, resulting in increased mucosal inflammation with the use of dentures\(^\text{(91,130-131)}\). Thus, healthy individuals as detected from their appearance and by their medical history, were selected to participate in this study. Moreover, none of the selected patients was receiving antibiotic or corticosteroid therapy during the whole follow-up
Discussion

period, which may affect the oral microbial balance \(^{(91)}\). The intake of some drugs as anticholinergics, antihistamines, sedatives and hypnotics have been shown to decrease salivary flow, providing a suitable environment for mucosal inflammation and infection \(^{(98)}\). Accordingly, patients receiving these drugs were not also considered.

Alteration of the oral microbial flora may occur in cases with extremely resorbed ridges, severe bilateral bony undercuts and thin or hyperplastic mucosa, as a result of denture trauma to the underlying mucosa. Thus, patients with the previously mentioned conditions were not included in this study \(^{(132)}\).

Inflammation of the oral mucosa has been reported to be associated with alteration in ph and secretion of inflammatory exudates, which enhance plaque accumulation and \textit{Candida albicans} growth \(^{(133)}\). For this reason, all cases were selected with clinically normal mucosa as a starting base line for this investigation. To avoid the cumulative effect of old dentures on plaque accumulation and \textit{Candida albicans} count, old denture wearers were not considered \(^{(106, 134, 135)}\).
Marked reduction in the frequency of yeasts isolated from smokers has been reported. Thus, all selected patients were non-smokers, to eliminate the possible effect of smoking on *Candida albicans* growth\(^{(136)}\).

The viscosity, composition and quantity of the salivary film, that exists between the denture base and oral mucosa directly influences denture retention. To avoid mechanical trauma resulting from loose dentures, patients with xerostomia, excessive salivation or thick ropy saliva were not considered\(^{(137)}\).

The concept of monoplane occlusion is concerned with the preservation of what remains, more than restoration of what is missing\(^{(138, 139)}\). Monoplane occlusion can adapt to the slight discrepancies between centric occlusion and centric relation that usually occurs due to processing errors of the acrylic denture base and/or settling of the dentures after wear. This prevents the mucous membrane from being crushed between the bone and the denture base, which may result in inflammation, pain, bone resorption or soft tissue hyperplasia. For the previous reasons, all dentures were constructed following the monoplane occlusion concept.

In this study, two commercially available heat
cured and microwave cured acrylic denture bases were selected for their clinical evaluation\textsuperscript{(140, 141)}.

Since the degree of curing affects the amount of residual monomer, degree of porosity, amount of stresses induced in the denture base and its dimensional stability\textsuperscript{(142)}. Dentures were properly cured following a long curing cycle (74°C for six hours).

The dentures were stored in tap water for 24 hours before delivery, to give chance for any residual monomer to be leached out, thus decreasing the likelihood of mucosal irritation\textsuperscript{(143, 144)}. Also, water sorption can be compensated for part of the processing shrinkage that usually occurs in acrylic resin dentures\textsuperscript{(142)}, thus helps in improving fit of the denture which may be a factor in plaque accumulation and microbial colonization.

At time of denture delivery, pressure indicating paste was used to detect the presence of any pressure areas on the fitting surface of the dentures. Elimination of the detected pressure areas improves fit and adaptation of the dentures.

Poor denture hygiene has been established as a significant factor in the development of inflammation of
the oral mucosa\textsuperscript{(87, 92, 145)}. At time of denture delivery, patients were instructed to clean their dentures after each meal under tap water, no other mechanical or chemical means were used, which might affect plaque accumulation and its microbial flora. Also, wearing dentures day and night increases plaque accumulation and hence, increases the risk of developing denture stomatitis\textsuperscript{(91, 134)}. For this reason, patients were instructed to remove their dentures during sleep for 6-8 hrs. This will also give chance for tissue recovery and physiologic stimulation, which is important for the integrity of the oral tissues.

\textit{Candida albicans} was the only strain investigated in this study, because it has been found to be the most prevalence of all Candida species, both in healthy and diseased oral cavities\textsuperscript{(87, 122, 146)}. Also, it has been reported to be the most pathogenic member of Candida species, capable of adhering to epithelial cells and acrylic surfaces causing infection\textsuperscript{(84, 92)}.

Oral rinse technique was employed in this study; this technique was selected because it is simple, rapid, sensitive and direct for monitoring the degree of colonization of \textit{Candida albicans} on dentures that could be correlated with the clinical findings\textsuperscript{(87, 90, 93-94)}. 
Discussion

The results of this study revealed an increase in both the frequency of *Candida albicans* carriers and the density of Candidal colonization after denture use in all the studied groups, but in different degrees. Although the prevalence of Candidal colonies was found to be greater in the salivary samples collected after the insertion of heat-cured acrylic resin compared to that after insertion of microwave-cured acrylic resin denture base, this increase was found to be insignificant. The increase of Candidal count is due to the fact that denture insertion causes changes in the ecology of the oral cavity and provides a saprophytic environment, which encourages *Candida albicans* growth. Also, the bacterial denture plaque that rapidly forms on the denture fitting surface promotes *Candida albicans* colonization. Moreover, the inevitable presence of porosities and roughness in the fitting surface of the dentures favor the mechanical retention of denture plaque to which *C. albicans* adhere, with subsequent colonization.

Several trials have been made to improve the surface quality of the denture fitting surface, which include, the use of improved type of stone for pouring the master cast, the use of tin foil as a separating medium, polishing of the fitting surface of the denture
and the application of denture glaze\textsuperscript{(105, 142)}. In spite of all these trials, there was always some degree of surface roughness and micro porosities that could not be completely overcome.

Although all selected subjects participating in this study were new denture wearers and had clinically normal healthy oral mucosa, \textit{Candida albicans} was isolated from almost 50\% of all participants before denture insertion. This can be considered within the range reported from 17\% to 60\%\textsuperscript{(100)}. The presence of \textit{Candida albicans} in some individuals and its absence in others can be attributed to individual variations, interactions with the normal bacterial flora\textsuperscript{(118)}, and the possible resistance of the mucosal surfaces of non-carrier individuals to \textit{Candida albicans} colonization\textsuperscript{(84)}.

The difference in the degree of \textit{Candida albicans} colonization between conventional heat-cured acrylic resin and microwave-cured acrylic resin was most probably due to the different physical properties of the materials, which determine the pattern of \textit{Candida albicans} adherence and hence its density of colonization.

The least Candidal count score encountered in the studied samples one month after rebasing the
dentures with microwave-cured acrylic resin could be attributed to its physical properties. Polymerization by microwave energy causes reduction in the internal and external porosity of the denture base. The high Candidal count score detected after rehabilitation of heat-cured acrylic resin could be attributed to the more surface porosity in the acrylic resin which in turn encourages harboring of Candida.

In addition Microwave-cured dentures were reported to have better dimensional accuracy than the conventional heat-cured ones. Movement of the heat-cured acrylic resin dentures during function and the release the residual monomer might induce traumatization and inflammation of the mucosa supporting the denture. The traumatized mucosa most probably increased the susceptibility of the tissues to Candidal infection and plays a role in the adhesion of Candida to the mucosal surface. This can also account for the increase in the Candidal colony score after rehabilitation the patients with heat-cured acrylic resin dentures base.

The increase in the degree of *Candida albicans* colonization recorded in the two groups might have been accentuated by the oral and denture hygiene
Discussion

regimen followed in this study. Brushing and chemicals that are sometimes used for denture cleansing were not used in this study, soft brushes were used gently and denture cleansers were used with suitable concentration, different results might have been recorded.

Scanning electron microscope photography sampled from patient no.5 after one month of wearing the conventional heat-cured complete denture revealed that, different budding forms (mono-polar, bi-polar and tri-polar) as shown in fig. (17), while scanning electron microscope photography sampled also from patient no.5 after one month of wearing the microwave-cured complete denture revealed that, in addition to the budding forms, formation of germ tubes (GT) and pseudohyphae with mucilaginous layer as shown in fig. (18), that is show how Candida albicans try to adapt to the new condition by formation of pseudohyphae which in turn gives off many budding and also the appearance of mucilaginous layer that Candida albicans secretes to enhance its adhesion to the microwave-cured complete denture, this is because curing by microwave brings smooth surface to the denture with less internal and external porosities, in contrast the conventional heat-cured complete denture, is a denture of rough surface containing a degree of porosities that allow Candida
Discussion

*Candida albicans* to adhere and inhabitant without facing a problem and that’s also why the number of Candidal count colonies counted on Sabouraud’s dextrose agar medium sampled from patient wearing conventional heat-cured complete denture was greater than those counted in case of microwave-cured complete denture.

Nevertheless, it should be noted that the greater count numbers of *Candida albicans* present in conventional heat-cured complete denture doesn't only represent the *Candida albicans* in pathogenic form but also, it might represent the non-pathogenic form.

Also, the numbers of the pathogenic forms are either of lesser virulence or have not reached the appropriate concentration needed for disease development and formation of symptoms characterizing Candida infection.

This speculation based on failure of detecting any inflammatory signs in the selected patients before and after insertion of the tested complete dentures.

In case of scanning electron microscope photography sampled from patient no.7 after one month of wearing the conventional heat-cured complete denture revealed that, different forms of budding and
Discussion

pseudohyphae formation as shown in fig. (19), while scanning electron microscope photography sampled also from patient no.7 after one month of wearing the Microwave-cured complete denture revealed that, only budding forms as shown in fig.(20).

It can be argued that the produced Candida albicans patterns is due to the condition of the Environment (health status of the patient), that’s if any condition threaten its survival, Candida albicans enter a phase of budding or pseudohyphae (which in turn gives off budding) that to ensure its existence.

Therefore, we can say that the health status of the patient might be a crucial factor among other factors examined in this study and as mentioned earlier in the only observed pattern of Candida albicans in the form of budding formation and in determining the behavior of Candida albicans for adaptation and survival.

To ascertain of the nature of pathogenicity and the level of virulence of this present Candida, further investigations should be carried out using the recent molecular-based diagnostics such as polymerase chain reaction(PCR) finger printing(RAPD-PCR) and reverse transcriptase polymerase chain reaction (RT-PCR).
V. Summary and conclusions:

This study was conducted to evaluate the effect of heat-cured acrylic resin denture base and microwave-cured acrylic resin denture base on Candidal growth.

Seven completely edentulous male patients with no history of denture wearing participated in this study. All the selected patients were re-habilitated by mucosa supported complete dentures.

The dentures were constructed from conventional heat-cured acrylic resin denture base following monoplane concept of occlusion. Before dismissing the patients and one month after denture insertion, salivary samples were collected according to oral rinse technique. One month resting period was allowed so as Candidal count can reach to normal. Then dentures were rebased using microwave-cured acrylic denture base, before denture insertion and one month after denture insertion, salivary sample were collected before and one month following the same oral rinse technique.

In the oral rinse technique, the patients were instructed to rinse their mouths with 10mL of sterile phosphate buffered saline for 60 seconds. The rinse was then expectorated into a universal container and immediately transported to the
laboratory for concentration by centrifugation, then cultured on sabouraud’s dextrose agar plates which were incubated at 37 °C for 48 hours.

Microscopic examination and germ tube test were carried out for laboratory investigations. In addition, the morphological features of the isolated Candida from the samples tested in this study, were investigated using the scanning electron microscope(SEM).
Conclusions

The results obtained from this study revealed that:

(1) Both the conventional heat-cured and microwave-cured acrylic resin dentures bases have the affinity to support *Candida albicans* growth.

(2) The microwave-cured acrylic resin denture base has the least affinity to *Candida albicans* colonization.

(3) The health status of the patient is of a crucial factor among other factors examined in this study in determining the behavior of *Candida albicans* for adaptation and survival.
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تأثير الأطقم الكاملة من الراتنجات المطبخة بالموجات الصغري على نمو فطر الكاندیا.

دراسة مقدمة إلى كلية طلب الأسنان جامعة عين شمس كجزء متم للحصول على درجة الماجستير في الاستعاضة الصناعية في طب الأسنان.

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الملخص العربي

يهدف هذا البحث إلى مقارنة نمو فطر الكانديدا البيضاء على نوعين مختلفين في طريقة الطيح من الأطقم الكاملة البلاستيكية.

في هذه الدراسة تم اختيار عدد 7 مرضى من عيادة قسم الاستعاضة الصناعية بكلية طب الأسنان جامعة عين شمس.

- تم عمل أطقم كاملة لكل المرضى على مرحلتين:
  - المرحلة الأولى: تم عمل أطقم كاملة مصنوعة من الراتنجات الحرارية الطيح.
  - المرحلة الثانية: تم تغيير القاعدة البلاستيكية لأطقم مصنوعة من الراتنجات مطبوعة بالموجات الصغرى.

وعند استلام المرضى للأطقم تم اخذ عينة من الفم وفحصها مجهرياً للتأكد من وجود أو خلوها من فطريات الكانديدا المسببة للالتهاب الغشاء المخاطي الملائم للطقم.

- وبعد ذلك، تم عمل التعديلات اللازمة على الأطقم المسلمة لهم، تم أخذ عينات من كل مريض بعد شهر من استلامه الطقم المصنوع من الراتنجات المطبوعة حراريًا.

- في هذه المرحلة تم توصية المرضى بعدم استخدام الأطقم لمدة شهر حتى تعود فطريات الكانديدا لمعظمها الطبيعية.

- تم أخذ عينات من كل مريض بعد شهر من استلامه الأطقم المصنوع من الراتنجات المطبوعة بالموجات الصغرى.

وفي نهاية كل فترة متابعة تم أخذ عينة وراعيتها على وسط مناسب (ساب وديكستروز أجار).

(...اسبوع وديكستروز أجار)...
تم فحصها مجهرياً بالميكروسكوب الضوئي للكشف عن وجود أي نمو للفطريات الكانديدا على هذه الأسطح في المرحلتين.

- تم استخدام الميكروسكوب الإلكتروني الماسح لتوضيح الخصائص المورفولوجية للكانديدا في العينات المختبرة محل الدراسة.

- أوضحت النتائج بأن العزلات تمثل الكانديدا الغير مرضية استناداً إلى عدم مصاحبتها لأي عرض من أعراض التهاب الغشاء المخاطي الملصق للطقم المميز للكنديدا المرضة.

- تم توضيح خصائصها المورفولوجية باستخدام الميكروسكوب الإلكتروني الماسح.

- تم مناقشة النتائج التي توصلت إليها الدراسة، استناداً إلى استخدام كلاً من الميكروسكوب الضوئي والميكروسكوب الإلكتروني الماسح لتمييز الخصائص المورفولوجية لعزلات الكانديدا من العينات المحززة محل الدراسة.

- تم مناقشة النتائج في ضوء التوصية بإجراء دراسات مستقبلية مستندة إلى الطرق الحديثة ذات الارتباط باستخدام تقنيات البيولوجيا الجزئية كوسائل تشخيصية وتأكيدية للتشخيص والتعريف الدقيق لعزلات الكانديدا محل الدراسة وارتباطها بنوع التركيبات التي تم دراستها.