Effect of Biofilm on the Mechanical Properties and Repair Strength of

Denture Acrylic

BY

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THESIS

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GKM
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<tr>
<td>ADA</td>
<td>American Dental Association</td>
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<td>ANOVA</td>
<td>analysis of variance</td>
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<td>C</td>
<td>Celsius</td>
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<td>DI</td>
<td>deionized</td>
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<td>H$_2$O</td>
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<td>N</td>
<td>Newtons</td>
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<tr>
<td>PMMA</td>
<td>Poly methyl methacrylate</td>
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<tr>
<td>p.s.i.</td>
<td>pounds per square inch</td>
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<tr>
<td>SDA</td>
<td>Sabouraud dextrose agar</td>
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<tr>
<td>SDB</td>
<td>Sabouraud dextrose broth</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscopy</td>
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<td>SiC</td>
<td>Silicon Carbide</td>
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SUMMARY

The purpose of this study was to investigate the effect of *Candida albicans* on the mechanical properties and repair strength of a standard denture acrylic, Lucitone 199® with standard repair resin. The goal was to determine the effect of exposure to this common oral fungal pathogen on denture wearers had on the mechanical properties of denture acrylic.

Standardized samples of Lucitone 199® denture acrylic were flaked and processed by traditional flask and fast cure methods according to the manufacturers specifications. Samples were trimmed and polished, and immersed in deionized H₂O at 37°C for 7 days. Samples were then divided into three groups and exposed to *C. albicans*. A group of control samples were used for baseline measurements, and experimental samples were exposed to *C. albicans* for 3 and 12 days, respectively. Mechanical testing of samples microhardness, surface roughness, and repair strength were tested before and after biofilm formation. All samples were tested for repair strength by 3-point bend after repair with Lucitone® repair resin according to the manufacturers specifications.

Descriptive and statistical analyses were performed on all data (SPSS v. 20, Armonk, NY, USA). The presence of biofilm produced by *C. albicans* significantly affected (p<0.05) the repair bond strength and microhardness of denture acrylic at both exposure intervals. Although compared to the untreated control, surface roughness of denture acrylic was not significantly affected (p>0.05), the p-values indicate a general trend towards increasing roughness with exposure time to *C. albicans* (3 days p=0.08, 12
days p=0.054). Data obtained from this study is important to the clinician, as the decrease in mechanical properties must be noted when deciding if repair or replacement of the prosthesis is warranted. Further research is needed in the depth of penetration by *Candida* biofilm *in vivo* to ensure adequate measures are taken by the clinician to avoid a decrease in repair resin mechanical properties. Studies on effective removal of biofilm from dental materials surface before manipulation is necessary. Research into *C. albicans* colonization and growth and how this relates to clinical exposure levels is needed to give definitive clinical recommendations.
1. Introduction

1.1 Background

There are estimated to be almost 60 million patients missing teeth in at least one complete arch of their dentition in the United State of America currently. The most common treatment modality for these patients is an acrylic based complete prosthesis. The highest complication rate associated with acrylic based prosthesis is acrylic fracture and tooth debonding. This occurs at varying rates ranging from 20-50% of prosthesis during their lifetimes.

Dental implants have become a common treatment modality, and the first choice treatment when rehabilitating the edentulous mandibular arch. Implants have shown to greatly improve quality of life in patients due to the increased comfort with removable prosthesis. One major reason for this is the increase in masticatory force one is able to place with the aid of implants. Acrylic is often integrated in implant prosthesis, especially in edentulous patients due to its ease of application and low cost.

Oral bacteria frequently colonize on removable prosthesis only a few days after delivery to the patient. Patients with acrylic prostheses are often lacking in their home oral hygiene, allowing these microbes to colonize and flourish in the nutrient rich oral environment. This may lead to denture related stomatitis, which is quite prevalent, often as high as 80% in the denture wearing population. The most common oral microorganism associated with acrylic prosthesis is Candida albicans. Its presence
and formation of biofilm can be expected to have many undesirable mechanical and systemic outcomes.

Previous research has evaluated how microbial biofilms affect composite repair, however, limited research exists on acrylic based materials. (10) With the large prevalence of *C. albicans* in edentulous patients, and the high rate of complications, it can be inferred that this may be one reason denture repairs may be compromised. Extensive research has been performed as to how to maximize effectiveness of denture repair, however, less emphasis has been placed on research investigating the effect of *Candida* biofilm on the repair strength of denture acrylic.

### 1.2 Significance

The increasing number of edentulous arches in the world’s population means the number of acrylic prosthesis in function continues to rise. Complication rates are high, and with the rise in popularity of dental implants to support and retain these prostheses, patients and clinicians will continue to see complications. Limited research has evaluated bonding surfaces and interfaces, and the effect of *C. albicans* on the repair strength of denture acrylic.

Previous research has shown a negative effect of the presence of oral biofilm in the repair strength of dental composites. (10) With the high prevalence of oral biofilm present on acrylic prosthesis, future research may be performed to aid clinicians in making educated decisions when faced with acrylic prostheses complications. The
clinician must decide if the prosthesis should be repaired or replaced. In this respect, the understanding of the effect of oral biofilm on the repair properties is essential. This study represents an initial step in identifying a problem with oral biofilm on repair strength. Future research into effective means to facilitate oral biofilm removal from denture surfaces, and to modify bonding surfaces to increase repair strength are warranted.

1.3 Specific Aims

The specific aim of this study was to: investigate the effect of the presence of of *C. albicans* biofilm on the repair strength of denture acrylic; mechanical properties of denture acrylic; and flexural strength of unrepaired denture acrylic.

1.4 Hypotheses

The hypotheses for this study was: oral biofilm of *Candida albicans* negatively affects the repair strength, the microhardness, and the surface roughness of denture acrylic.
2. Review of Literature

2.1 Edentulism

Edentulism is defined as total tooth loss. Edentulism is most prevalent among those lacking a high school education, people lacking dental insurance, non-Hispanic blacks, and everyday smokers. Rates of edentulism vary worldwide and presently are approximately 30% in the United States. Between 1950s the 1990s, rates of edentulism in the United States displayed a decrease from 50% to 42% in the age categories over 65. The rate of edentulism also decreased from 28% to 11% in the 45 to 64 year old age demographic, and decreased from 5% to 2% for those 18 to 44 year old demographic.

Douglass et al. (2002) examined the need for complete dentures currently and going forward. Although the rates of edentulism are declining, the population is rapidly increasing, resulting in a steady increase in patients requiring prosthetic care to replace missing teeth. It was estimated that in 2010, 59.2 million edentulous arches existed in the United States, and this number may climb to 61 million by 2020. Treatment of these patients poses many issues for dental providers, especially with material choices.

2.2 Denture Materials and Properties

Poly methyl methacrylate (PMMA) resins have long been the standard denture material. The two most common PMMAs used for denture fabrication are heat cured...
poly(methyl methacrylate) and rubber-reinforced poly (methyl methacrylate). A majority of dentures fabricated today are made from these two materials. These materials offer impact resistance, strength, and toughness to withstand the mastication forces found intraorally.

Lucitone 199® (Dentsply International Inc., York, PA, USA) is a heat activated denture base. Thermal energy is required for polymerization, which usually is provided by a water bath. Lucitone is composed of a powder and liquid. The powder is prepolymerized spheres of PMMA and benzoyl peroxide, which serves as an initiator. The liquid is predominantly non-polymerized methyl methacrylate and hydroquinone, an inhibitor. A polymer to monomer ratio of 3:1 by volume is generally used.

Denture repair resin (Dentsply International Inc., York, PA, USA) is a chemically activated denture base resin. It is polymerized at room temperature, and is often referred to as an autopolymerizing resin. The monomer is similar to that of heat activated denture base, but a tertiary amine such as dimethyl-para-toluidine is added. This causes a decomposition of the benzoyl peroxide inhibitor and allows autopolymerization.

2.3 Denture complications

PMMAs are not without complication in clinical use. Bilhan et al. (15) examined 99 patients who had been wearing some form of acrylic prosthesis for 3-5 years. Patients were examined by 2 prosthodontists and their protheses rated. Complication rates with PMMA prosthesis were 28% for denture fracture and 32% for tooth debonding.
Dentures fracture for many reasons. Beyli et al. (16) evaluated the causes of denture fracture. PMMA materials deform during function, resulting in increased stress and strain, especially in a maxillary prosthesis. Dentures contain stress within due to bending deformation and tensile stresses. Midline fracture is the most common failure within acrylic and is a result of cyclic deformation of the denture base during function.

Morris et al. (17) also discussed flexural strength of maxillary denture bases. A denture may be subject to >500,000 cycles of flexion from mastication a year. The authors attempted to examine different anatomical considerations present in a maxilla and the reason denture bases fractured. They determined that a shallow palatal vault, most commonly seen in the severely resorbed maxilla, is most susceptible to fracture.

Darbar et al. (18) surveyed British dentists documenting fracture type and complication rates of dentures. Of all complications, 33% were debonded/detached teeth, 29% were midline fractures usually in Maxillary complete dentures, and the remaining 38% were other types of fractures, usually to Maxillary partial dentures. Most these repairs can be completed chair side, often with an autopolymerizing resin.

Goodacre et al. (2) evaluated clinical complications with implants and implant prosthesis. Complications must have been present in 3 or more studies for inclusion, and percentages of complication rate were reported. Common mechanical complications included resin veneer fracture of fixed partial dentures (22%), overdenture fracture
(12%), opposing prosthesis fracture (12%), and acrylic resin base fracture (7%). Incidence of implant born prosthesis complication was higher than that of natural tooth.

With the rise of oral implants, mastication forces on acrylic prosthesis has greatly increased, leading to the possibility of greater risk of fracture of acrylic based materials. With this rise in complication rate, further research will need to be performed to determine repair options and properties. The effect of many confounding factors will influence the method of acrylic repair.

Bozini et al.(19) performed a systematic review of the literature to determine complication rates of implant supported prosthesis. Nineteen total studies were reviewed and a meta analysis performed. The most common complications are veneer fracture and material wear, both requiring repair. Most studies were of mandibular implant retained prosthesis opposing complete dentures.

Priest et al.(20) examined prosthetic complications in metal acrylic resin implant borne complete fixed prosthesis over a 22-year period in a private practice setting. Forty-five patients were in the study. Twenty-nine percent of the patients had to have teeth repaired/replaced over the timeline of the study, and most issues with the teeth were a result of opposing a fixed dentition.

Lindquist and Carlsson(4) evaluated the effect of chewing on mandibular fixed prosthesis. Patients were given an optimal conventional denture first, followed by a fixed
prosthesis on osseointegrated dental implants, both in the mandible. Patients were evaluated by a questionnaire, a comminution test of chewing efficiency, and bite force measurements on 4 occasions: with original dentures, then optimal dentures at delivery, 2 months, and 3 years of follow up. Conventional dentures resulted in no improvement in chewing efficiency, however implant supported prosthesis continued to observe improvements the longer the patient had the prosthesis. The authors concluded that patients show increased bite force with implant supported prosthesis, and also that patients increase bite force with time while adapting to a fixed prosthesis.

2.4 *Candida albicans*

*Candida albicans* is a dimorphic yeast that is most prevalent in immunocompromised patients, the elderly, or patients undergoing immunosuppressive therapy. Most individuals carry only one strain of *Candida*, however some patients may carry multiple strains. The microbe is most commonly found in the gastrointestinal tract and in oral flora. If the number of *Candida* microbes increases over a certain threshold, it may spread to other sites in the human body, causing fungaemia and funguria.(21)

*Candida* can reproduce in two different ways, by budding to form an ellipsoid bud, or by hyphal reproduction, periodically fragmenting to give rise to new mycelia. Initially blastospores start as unicellular forms of fungus that divide by budding. In the presence of certain environmental factors, cylindrical outgrowth is initiated on the blastospore to form a germ tube. Germ tubes grow at the apical tip to form hypha. These hypha branch, and lay down more septa that eventually form mycelium. Secondary blastospores may become separated from the filament to form their own colony.(22)
Candida metabolism has been shown to release several acids during metabolism.(23) Candida has been isolated in dentate patients in carious dentin lesions. This may be due to the inherent ability of the microbe to produce and withstand an acidic environment.(24) The presence of acid associated with C. albicans has been shown to have a negative effect on dental composites and denture acrylics.(10,25)

Candida infections are most commonly treated with oral rinses, troches, or systemic medications. These include antimycotics, with antifungal drugs including topical cotrimazole or nystatin, fluconazole, or topical ketoconazole. Current meta-analysis data shows that when treating oral stomatitis reactions and those associated with Candida albicans, disinfection agents, antiseptic mouthwashes, microwave disinfection, and antifungal medications are all acceptable.(26)

2.5 Oral Microorganisms and Denture Acrylics

The oral cavity harbors a vast quantity of microorganisms in the human oral cavity. They form biofilms at great rates on hard and soft tissues, as well as biomaterials used commonly to restore function and esthetics.(27,28) Biofilm formation in vivo is present on the physical and chemical characteristics of a materials surface, such as hydrophobicity, charge, and roughness. Saliva further enhances biofilm formation due to mineral content and harboring of ideal growing conditions. However, dental materials provide the substratum for biofilm formation.(29)
The dentate and edentulous patients have different environments, and have been shown to harbor different microorganisms. Sachdeo et al. studied biofilms in the edentulous cavity. Biofilm samples were taken from 61 edentulous patients. Forty-one samples were tested for after biofilm growth. High numbers of *Actinomyces* species, *V. parvula*, and *Streptococcus* species including *Streptococcus mutans* were isolated. The biggest surprise of the study was the presence of *A. actinomycetemcomitans* and *P. gingivalis*, previously thought to only be associated to dentate patients with periodontal disease. The authors concluded that a large mix of microbes are present even in the edentulous patient, and are present throughout many different areas of the oral cavity.

Denture stomatitis is present in up to 70% of denture wearing patients. Diagnosis of denture related stomatitis is usually based on clinical findings. It is associated with removable prosthetic prosthesis that usually partially or fully covers the maxillary palate. Denture stomatitis etiology may be due to systemic disease, smoking, overnight denture wear, and poor oral hygiene. This is associated with *C. albicans* colonization on the denture surface. Denture stomatitis may also be caused by irritant or allergic reactions to denture base material, or by systemic factors such as hematologic disorders or a dietary insufficiency.

Barbeau et al. re-evaluated the link between *C. albicans* and denture stomatitis according to a modified Newton classification. They evaluated 68 subjects from 2 studies. Patients were part of prosthodontic clinics in university settings. Patients wore complete maxillary acrylic dentures. Patients were asked to complete a
questionnaire concerning their health condition and lifestyle habits. Patients fall into three categories of Newton classifications. Patients may have no stomatitis, Newton type I stomatitis which present with petechiae present on the palatal mucosa that may contact the denture surface. Newton type II stomatitis involves macular erythema without hyperplasia, and New Type II presents with localized or generalized erythema concurrently with papillary hyperplasia. Cohabitation of different Candida species was found more frequently in subjects with stomatitis compared to healthy individuals. Inflammation was associated with stomatitis, and wearing the denture at night and smoking increased inflammation.

Webb et al. (36) reviewed the etiology and management of candida associate denture stomatitis. Candida infections are more likely in immunosuppressed individuals. Candida form soft, cream-colored colonies with a yeasty odor. They thrive at temperatures similar to mouth temperatures. Yeasts are gram-positive with shapes that vary from ovoid to elongated or spherical which transform to hyphae. C. albicans can be easily identified by pseudohyphae. Pathogenic candida species assimilate and ferment glucose for their primary carbon source. Saliva tends to promote adhesions of C. albicans to acrylic, thus growth may still happen but adhesion is limited.

Gendreau et al. (37) reviewed epidemiology and etiology of denture stomatitis. Etiological factors of stomatitis may include inadequate denture hygiene, excessive use and overnight wear of prostheses, or accumulation of plaque, bacterial, and yeast accumulation on the denture surface. Ill-fitting prostheses can also result in an increase
in mucosal trauma. These factors have been shown to enhance the ability of *C. albicans* to successfully colonize the surfaces of oral prostheses.

*Candida* is very effective at colonization in edentulous patients due to its ability to adhere to denture base materials. Koch *et al.* 2013(9) examined the ability of *Candida* to adhere and colonize on six different common denture base materials. After thermocycling of the materials and incubation with *C. albicans* for either 24 or 196 hours, the amount of fungus was measured. Koch concluded that *C. albicans* was much more successful at colonizing denture materials that were polar with high surface energy. This supported the hypothesis that there may be a relationship between surface free energy and *C. albicans* proliferation.

A comparison was done by Teles *et al.*, researching the different microbes present in dentate versus edentulous patients, and the rebound of microbes in these two patient groups after professional cleaning. Supragingival plaque and biofilm samples were taken from 55 dentate and 62 edentulous patients denture teeth before and after professional cleaning. The paper found higher levels of *Streptococcus mitis*, *Streptococcus oralis*, and *Streptococcus mutans* in edentulous patients, whereas dentate patients had higher levels of *Tannerella forsythia*, *Selenomonas noxia*, and *Neisseria mucosa*. After cleaning, dentate patients had a faster and higher rebound of microbial load, however after full recovery dentate and edentulous patients have similar total numbers of total bacteria, but in different proportions.
Riberiro et al.(7) examined 90 denture wearers with healthy mucosa and without a presence of stomatitis. Microbial swabs were collected from each denture and colonized in an attempt to determine what microbes are typically living on the surface of patient’s dentures. Candida species were found in 65.6% of the dentures, compared to 53% for Streptococcus mutans and 34% for Staphylococcus aureus. This confirmed previous research and the prevalence of Candida in edentulous patients. Most denture wearers are elderly, and the constant ingestion and aspiration of microorganisms from denture plaque exposes these individuals to extra risk of unwanted disease and illness.

Oral candiosis is more prevalent in immunocompromised patients. Budtz-Jorgensen et al.(38) examined a cohort of 233 patients, of which 146 were edentulous in at least one arch. Seventy two percent of the patients wearing dentures had denture stomatitis, and yeast counts were significantly correlated with the intensity of erythema of the palatal mucosa. The conclusion was that high oral yeast counts and prevalence of oral candiosis of institutionalized patients was associated with poor oral hygiene and a neglect of denture care.

Denture liners have also been associated with microorganism growth. Makila et al.(39) examined 39 denture wearers with Molloplast® liner in the mandible and conventional heat processed denture in the maxilla. Fungal growth was observed in 85% of mandibular dentures and 44% of maxillary dentures. This was a statistically significant difference. Patients who had inflamed tissue also were associated with fungal
growth. The presence of liners greatly increased the frequency of fungal, and more specifically *Candida albicans* infections.

Wright *et al.* (40) found similar findings, examining 53 denture wearers with soft lined mandibular dentures opposing maxillary acrylic resin dentures. Yeasts were identified in 66% of the subjects, with *C. albicans* occurring either alone or with other species was isolated in 66% of the patients. An association was found between denture cleaning, hygiene, smoking habits, and the subsequent prevalence of yeasts.

Graham *et al.* (41) evaluated 2 different soft denture liners in 14 edentulous patients for one month after placement. Cytologic smears were made on the liner surface at 1 hour, 1, 2, 7, 14, and 30 days after intraoral placement. Six patients from one material showed yeast formation and 2 from the other group. A total of 8/14 (57%) of the patients demonstrated yeast formation in the growth period. Graham and colleagues concluded that soft liners should only be used for short time periods intraorally, they should not be used to treat candidiasis, and that there was no significant difference in the prevalence of fungal growth between the two liners tested.

Several studies have attempted to place antimicrobial additives into denture liners to control microbial growth. Lefebvre *et al.* (42) mixed microban, a broad-spectrum antimicrobial triclosan, with PermaSoft denture liner. The goal was to determine if the addition of microban would inhibit the growth of *C. albicans*. This bench top study
studied the cytotoxic effect over a 24-hour period. There was no statistically difference in the *Candida* growth in PermaSoft with or without Microban added.

Bueno *et al.* (43) added nystatin, miconazole, ketoconazole, itraconazole, and chlorhexidine diacetate to two different soft denture materials, Trusoft and Softone. After 14 days of incubation, it was determined that more than 90% of fungal growth was inhibited compared to controls. This in vitro study shows promising results, however the concentration of drugs released by miconazole and itraconazole was over the daily recommended dosage, so the elixir may prove deadly in vivo.

Others have evaluated the addition to magnesium oxide as an inhibitor of *C. albicans* growth. Kanathila *et al.* (44) examined the effectiveness of magnesium oxide with Viscogel tissue conditioner in vitro. Concentrations of 1%, 5%, and 7% magnesium oxide were tested. After 24 hours, 1% showed no statistical difference with control at *C. albicans* growth inhibition, but 5% and 7% did. However the authors could not conclude if the concentrations tested would be biocompatible when placed in vivo.

Prevention of stomatitis can be aided by proper home hygiene. Unfortunately, many patients never even receive proper hygiene instructions. Marchini *et al.* (45) surveyed 236 complete denture wearers who were at least ten years post insertion of prosthesis. In this sample size, 77.5% reports they had never been given instructions about hygiene of their dentures, 91.9% were never told to return for periodic reviews, and only 43.6% had returned for dental treatment at least once in the previous 10 years.
Further, a positive relationship was found between lack of cleansing recommendations and presence of denture-related stomatitis and hyperplasia. Other factors related to complications with oral prosthesis were family income and periodicity of dental visits.

2.6 Denture repair

Once a denture fractures, its repair is desirable because it is expensive and time consuming for both patient and provider to construct a new prosthesis. Polyzois et al. (46) classified acrylic resin denture repair and its effect on strength parameters. Maxillary midline fracture is the most common complication, followed by mandibular midline fracture. Reasons for fracture are due to excess stress and bending at the midline. A satisfactory repair must have adequate strength, be easily and rapidly completed, match the original color, and retain dimensional accuracy. In this bench top study wire reinforcement was added to repairs. This may reduce flexure of the prosthesis and increase repair strength. The study concluded that every clinical situation is unique and different, however results of the study show that adding wire reinforcement may increase repair strength.

Once a denture has fractured, there is common agreement that the repair strength will never match the original fracture strength and toughness. The repair of denture can be done by several different methods. Alkurt et al.(47) evaluated the effect of repair resin type, surface treatment, and repair strength on heat polymerized denture base. Methods of acrylic repair include heat-polymerized acrylic resin, autopolymerizing resin, and light polymerized resin. All are easily completed in one chairside visit. Repair
strength for autopolymerizing repair resins can be 55% or less of original denture strength. Additional methods of preparing the denture surface before repair were also considered. These included treatment of the surface with methyl methacrylate monomer, airborne particle abrasions, and erbium:yttrium aluminum-garnet laser. All treatment groups exhibited and increase in repair strength, with heat-polymerized resin being of higher flexural strength that autopolymerized and light-polymerized resins. Airborne particle abrasion showed the highest increase in flexural strength.

Seo et al.(48) reviewed the factors that affect denture repair. Repair material selection can affect repair strength, as autopolymerizing are reported at 60-65% of repair strength, whereas heat polymerized repair resins may have 75%-80% of original bulk material strength. The heat polymerized is less desirable in practice due to extra laboratory steps, cost, and extended time. Light polymerized resins may be used, however they display increased water sorption, poor adhesion to denture teeth, increase brittleness and reduced impact resistance. The repair with light polymerized materials may be easy for clinician to handle, however they lack the same properties autopolymerizing resins do.

Seo et al.(48) also reported that reinforcement with wires, nylon, or fibers may improve repair strength. These studies were often coupled with surface treatments, most commonly air abrasion. Other surface treatments include chemical treatments such as wetting with methacrylate monomer, chloroform, acetone, or methylene chloride. These agents may help clean the surface and also cause pitting, increasing surface area for
repair bonding. Mechanical treatment of the interface can include placement of bevels, butt joints, or rabbet joint. These increase surface area and micromechanical interlocking. The gap of repair can range from 1.5-10mm, with agreement that in vivo a 3mm gap is best for adequate material thickness while decreasing the amount of polymerization shrinkage.

An alternative repair material is microwaveable acrylic resin. Ng et al.\(^{(49)}\) compared the fracture toughness and shear strength of microwaveable acrylic repair resin compared to common autopolymerizing repair materials. A benchtop study was performed and found that bond strengths were equal to or stronger than industry standard autopolymerizing repair materials. Ng and colleagues concluded that microwave cured resins are viable alternatives for denture repair.

Further methods of acrylic/tooth preparation are available to increase the bond strength. Meng et al.\(^{(50)}\) evaluated 4 different treatment methods of denture teeth to increase the repair strength of denture teeth to denture base. These included grinding, sandblasting, placement of a diatoric, and treatment with a novel methacrylate monomer. It was concluded that placing a diatoric and treatment with a monomer were the most effective in increasing tooth bond strength by up to 3 times over conventional grinding.

The effect of biofilm on denture base has only been studied by one previous research group. Sahin et al.\(^{(25)}\) evaluated the effect of biofilm formation and biocorrosion on denture base fractures. Three different common heat polymerized
materials were used. Samples were exposed to 6 different microorganisms and the biofilm penetration, SEM of the surface, and fracture strength were calculated. Significant differences were seen in adhesion between organisms to the denture base, and a decrease in value in three-point bend after exposure to biofilm.

The frequency of complications with acrylic prosthesis, combined with the presence of many microorganisms poses interesting problems for the clinician when problems are encountered. Rinastiti et al. (10) examined the effect of oral biofilms on the repair strength of composites. The goal was to examine if the presence of an oral biofilm would affect the bond strength of composite-to-composite. When composites fracture intraorally, the decision of repair vs. replacement must be made. A significantly lower bond strength was observed after exposure to biofilm in vitro. The conclusion was that the presence of biofilm does affect bond strength, and the clinician must take this into account when repairing composite materials.
3. Methodology

3.1 Study Design

A total of 90 denture acrylic samples were fabricated according to the manufacturers recommendations. Samples were fabricated from a standardized acrylic jig (60mm x 5mm x 3mm). The standardized jigs were placed in Type III dental laboratory stone (GC America, Alsip, IL, USA) and flanked in a denture flask (Hanau Type, Whip-Mix Corporation, Louisville, Kentucky, USA). Acrylic was processed following a fast cure time interval (90 minutes 163°F, 30 minutes 212°F). Samples were deflasked and gross trimmed using a carbide bur (Brassler USA, Savannah, GA). Samples were then polished using 200, 400, and 800 grit sand paper by rotary instrumentation. Samples were measured with digital calipers (Model 147, General Tools and Instruments, New York, New York) to confirm samples were within 0.1mm of the desired dimensions. Upon completion of sample preparation, all samples were immersed in deionized (DI) H$_2$O at 37°C for 7 days. Samples were randomly divided into 3 groups (n=30). Each group of 30 was divided into 2 groups of 12 and one group of 6. Samples were randomly selected from each group to form their respective sub groups.

Samples then went through microhardness and surface roughness testing to establish control and baseline values. Twelve samples from each group were sectioned with a 5mm section removed from the middle, and 12 samples were left fully intact. Two of the three groups were then cold sterilized by ethylene oxide gas and subject to C. albicans biofilm growth for 3 and 12 days respectively. After biofilm growth, samples
were disinfected with Cavicide® for 5 minutes (Metrex Research, Romulus, Michigan, USA).

After disinfection, samples were retested for knoop microhardness and surface roughness. Samples had been marked so the same area of each sample was tested before and after *C. albicans* biofilm exposure.

Sectioned samples were repaired with autopolymerizing acrylic repair resin. Samples were gross trimmed with a carbide bur to remove any extra flash. All samples were placed in a standardized jig and tested for flexural strength to failure by 3-point bend test. After failure, samples were examined to determine the nature of the failure. SEM was done to examine biofilm growth and failure type.

### 3.2 Methods and Materials

**PMMA MATERIAL**

The denture base material selected in this study was Lucitone 199® (Dentsply International Inc., York, PA, USA). This material was chosen as it is the most commonly used denture acrylic used for many different prosthesis in dentistry.
SPECIMEN GEOMETRY

**Flexural Strength:** Templates of standard size were fabricated 60mm x 5mm x 3mm. Half the samples were sectioned with a 5mm piece removed to allow for 5mm repair resin in the experimental samples.

**Microhardness:** Samples for this group were fabricated from a standardized jig of 60mm x 5mm x 3mm. The samples were then sectioned into 12mm increments to be tested for microhardness. Samples were marked in the center with a carbide bur. Five measurements were taken at marked points (Leco® Corp. M-400 St. Joseph, Michigan, USA) for every sample to obtain an average knoop microhardness.

**Surface Roughness:** Samples for this group were fabricated from a standardized 60mm x 5mm x 3mm jig. The samples were marked and divided into 8 segments. At each mark is where measurements occurred. Measurements were recorded at baseline and after biofilm exposure.

SPECIMEN FABRICATION

Ninety specimens were fabricated from Lucitone 199® (Dentsply Intl, York, PA) original shade according to the manufacturers specifications. Samples were flaked according to conventional flaking techniques using Type III stone (Coe Cal, GC America, Alsip, IL, USA), and spatulated in a vacuum mixer (30 seconds mixing time, 25
Samples were flaked in two layers with separator placed between layers (Al Cote, Dentsply Intl, York, PA) (Figure 1).

A powder/liquid ratio of: 45cc/15mL and mixing time of 15-30 seconds was used. The powder was added to the liquid monomer and mixed for 15 to 20 seconds, until all powder was wetted. The mix was covered and allowed to sit until the packing consistency was reached (approximately 9 minutes at room temperature of 73 +/- 2°F).

Separator (Al Cote, Dentsply Intl, York, PA) was placed on the flaked samples before packing. The mix was packed using conventional denture flasks (Hanau Type, Whip-Mix Corporation, Louisville, Kentucky, USA) and not exceeding 10 minutes of working time. Trial packing was completed at 1500 p.s.i., 2500 p.s.i., and the final pack at 3500 p.s.i. The flasks were closed and locked by spring clam, and cured with a fast cure 163°F ± 2°F for 90 minutes followed by 30 minutes of boiling. Samples were allowed to bench cool for > 45 minutes before deflasking. All samples were fabricated using the same bottle of polymer and monomer.

Specimens were gross trimmed with a carbide bur (Brassler USA, Savannah, GA) and then sequentially polished on a polishing machine using 240 grit, 600 grit, 800 grit SiC paper until a smooth luster was gained. Samples for surface roughness were polished to 1200 grit. Specimens were placed in deionized water for 7 days at 37°C to allow for maximum water sorption (Figure 2).
Specimens were divided into 3 groups: control, 3 days, and 12 days, and then again divided in half. Half the samples were left intact, and half were placed in a jig and sectioned, removing a 5mm segment.

Testing Summary:

Flexural strength:
- 2 sample types per group
- Sectioned sample (27.5mm x 5mm x 3mm X 2)
- Non-sectioned sample (60mm x 5mm x 3mm)
- Span width 50mm
- Repair resin length: 5mm
- Loaded in Instron machine at 5mm/min until failure

Microhardness
- sample: 12mmx 5mm x 3mm
- Notch in center of sample to ensure data collected at similar point
- 5 measurements per sample across linear area all 0.75mm apart
- Average gave knoop microhardness

Surface roughness
- Sample: 60mm x 5mm x 3mm
- Notched at 8 points were data collection would occur
Microhardness

Original samples were sectioned into 12mm in length pieces and notched in the center (6mm). These samples were then evaluated on a Knoop hardness tester (Leco® Corp. M-400 St. Joseph, Michigan, USA). A 100g load with a 15 sec. dwell time was applied to the sample 5 times to obtain an average knoop number. Testing was done at initial, and then the same samples tested after experimental exposure.

The Knoop hardness number (KHN) can be calculated by the ratio of load applied to the area of indentation: \[ \text{KHN} = \frac{L}{l^2 C_p} \] where \( L \) is the load applied in kgf, \( l \) is the length of the long diagonal of the indentation in mm, and \( C_p \) is a constant relating to \( l \) and the projected area of indentation. The units for KHN are kg/mm². The higher the knoop value, the harder the material is.

Surface Roughness

A white-light interferometry microscope was used to obtain three-dimensional images of acrylic surfaces (Zygo New View 6300, Zygo Corporation, Middlefield, CT, USA). Eight measurements were taken for each sample. Images were taken before and after \( C. \text{albicans} \) exposure on each sample in order to evaluate the physical changes to the acrylic surface. Two parameters for surface roughness were examined (Ra/arithmetic average and rms/root mean squared).

Colonization and Growth of Candida Biofilm on Denture Material

Samples were divided into groups and cold sterilized by Ethylene Oxide for 24 hours.
*Candida albicans* SC 5314 was aerobically cultured at 37°C for 24hr on Sabouraud dextrose agar (SDA), and a loopful of growth was inoculated into Sabouraud dextrose broth (SDB). After 18–20 hr of incubation, cells were washed twice with phosphate buffered saline, 0.05M, pH 6.8; (PBS), re-suspended in SDB, and standardized to 1×10^7 cells/ml spectrophotometrically (OD value of 1.4 at 550nm wavelength).

**Biofilm formation**

For colonization of *C. albicans* biofilm, the denture acrylic samples were placed into flat-bottomed 6-well tissue culture plates. Three samples were placed in each well. Aliquot of 4.0 ml of *C. albicans* suspension was transferred into each well and incubated for 3 hours (hr) at 37°C (adhesion phase). After the adhesion phase, the cell suspension was gently aspirated and each sample was washed twice with phosphate buffered saline. For further biofilm growth (biofilm phase), 4.0 ml of freshly prepared SDB was added to each well. All plates were incubated for 3 days and 12 days at 37°C aerobically. Growth media in each well was replaced every day. After every 24 hr of incubation, the medium in each well was aspirated and specimens were washed twice with PBS, followed by the addition of 4.0 ml of fresh medium.

**Sample disinfection**

After 3 and 12 days, samples were removed from wells, rinsed twice with PBS, soaked in Cavicide for 5 minutes (Metrex Research, Romulus, Michigan, USA.) and followed by PBS wash. Samples were then stored in deionized water until mechanical testing occurred.
Sample Repair

After disinfection, samples were placed in a standardized jig to ensure the proper spacing before repair. Samples were placed so a 5mm gap distance was present between them to be filled with repair resin. Samples were not treated in any way to mechanically remove any *C. albicans* biofilm or adjust the bonding surface. Denture autopolymerizing repair resin (Dentsply® Repair Material, Dentsply International Inc., York, PA, USA), was placed by a bead-brush technique. This technique is the most clinically applicable technique used and allows for an approximate powder:liquid ratio of 3:1. Upon filling of the 5mm gap with repair resin, the jig with sample was placed into a pressure pot (Presto Industries, Eau Claire, WI) and the samples were polymerized at 45°C and 20 pounds per square inch (p.s.i.) for 15 minutes per the manufacturers specifications. Samples were removed and gross trimmed with a carbide bur (Brassler USA, Savannah, GA) to remove any residual flash.

Flexural Strength

Flexural strength was determined using a three-point bending test (ISO specification 20795-1:2008). Three groups with two subgroups were prepared (control, 3 days biofilm exposure, 12 days biofilm exposure). The subgroups were repaired and unrepaired samples. The samples were tested in a universal testing machine (Sintech Renew 1121, Instron Engineering Corp., Canton, MA, USA). A standard three-point bending jig was placed in the machine and samples centered in the jig. The test was
carried out at a crosshead speed of 5mm/min with a 100kg loading cell (Figure 3). The maximum load at failure was reported by the machine.

**Mode of Failure**

All samples that had been repaired were evaluated with an optical microscope (SMZ-10; Nikon Corp, Tokyo, Japan) at 10X magnification in order to classify the failure between repair resin and native resin. An adhesive failure was one in which the resin failed to bond to the native acrylic. There was no repair material on the native acrylic bonding interface after failure. A cohesive failure was one where the sample failed either all in native acrylic or all in repair resin. A mixed failure was one where some residual repair resin was left bonded to the native acrylic resin.

**Scanning Electron Microscopy**

After growth of *Candida* biofilm on the acrylic surface, and fracture of samples, SEM was completed (Hitachi S-3000N). First, samples were fixed with hexamethyldisilizane, and dehydrated with multiple series of ethanol. The samples were then mounted on an aluminum stub with double-sided conductive carbon tape and coated with gold particles prior to SEM imaging.
3.3 **Statistical Analysis**

Statistical software (SPSS v.20, Armonk, NY, USA) was used for descriptive and statistical analyses. Comparisons were performed within groups (repaired vs. non-repaired) and among groups (control, 3 days biofilm growth, 12 days biofilm growth).

For flexural strength testing, ANOVA testing was used to compare repair strengths among similar groups (control vs. 3 days vs. 12 days biofilm exposure) of non-repaired samples. ANOVA testing was also performed among groups of repaired samples, (Control vs. 3 days vs. 12 days).

For microhardness testing, testing was performed for groups before and after *C. albicans* biofilm exposure. For the 3 day group, paired samples t-testing was done to compare microhardness values pre and post exposure. A paired samples t-test was also done between samples for the 12 day exposure group.

Surface roughness had initial values taken on a sample group, and then values taken on the same sample group after exposure to *C. albicans* biofilm. Paired samples t-test were performed for groups 3 days and 12 days.
Table 1

FLOWCHART OF RESEARCH PROTOCOL

90 Samples
(24 control, 24 - 3 days, 24 - 12 days, 18 misc)

37°C water bath for 7 days

Initial surface roughness, microhardness (18 samples)

24 samples
(12 sectioned, 12 control, 6 SEM)

Repaired

Fracture strength

24 samples
(12 sectioned, 12 control, 6 SEM)

3 days Incubation with C. albicans in Sabouraud dextrose broth 37 C° aerobically

Repaired

Fracture strength, microhardness, surface roughness, SEM

24 samples
(12 sectioned, 12 control, 6 SEM)

12 days Incubation with C. albicans in Sabouraud dextrose broth 37 C° aerobically

Repaired

Fracture strength, microhardness, surface roughness, SEM
4. Results

4.1 *Candida albicans* Biofilm Growth on Samples

*Candida albicans* biofilm growth was confirmed by visualization of the white appearing biofilm on both 3 day and 12 day samples. This was confirmed by SEM (Figure 4), which showed *C. albicans* hypha present in both biofilm growth times. The amount of biofilm was visually determined to be greater in density at 12 days than 3 days, which was also observed on SEM. In day 3, blastospores and germ tubes are present. At day 12, hyphae with secondary blastospores are observed.

4.2 Flexural Strength

Both the unrepaired and repaired samples were tested. Unrepaired samples showed no significant difference in flexural strength among control, 3 days, and 12 days of biofilm exposure (p> 0.05). Unrepaired samples had average flexural strength means of 46.2N (σ= 9.2) for control, 47.2N (σ= 5.84) for 3 days, and 44.5N (σ=7.56) for 12 days. No significant differences were found. Repaired samples showed a steady decrease in flexural strength, with the control having a mean of 14.4N (σ=6.57), 3 days 5.7N (σ=2.81), and 12 days 3.6N (σ= 2.50). Within groups, all had statistically significant means (p<0.05). A drastic decrease in sample strength was observed between control and repaired samples. Between group comparisons also showed statistically significant changes, as the repair strength greatly decrease from control to 3 days and 12 days of biofilm formation (P<0.001) (Figure 5). The repair strength significantly decreased with exposure to biofilm.
4.3 Microhardness

Samples were tested at baseline, and after biofilm growth of 3 days and 12 days. Significant differences in microhardness were observed from baseline to experimental. The overall microhardness of the samples decreased for both time intervals. In the 3 day group, the initial microhardness mean of 388.3 (σ=31.7), which decreased to 364.5 (σ=16.6). In the 12 day group, the initial mean of 402.4 (σ=42.3) decreased to 362.2 (σ=17.6). Both results were statistically significant (p< 0.05) (Figure 6).

4.4 Surface Roughness

Samples were tested before and after biofilm formation. Samples did show a general trend of increasing surface roughness from baseline, however the results were not statistically significant for 3 days (p= 0.08,) or 12 days (p= 0.056) (Figure 7). The roughness for 3 days had an initial value of 0.060 (σ=0.010) and a final value of 0.865 (σ=0.010). The 12 day samples had a starting roughness of 0.068 (σ=0.009), and a final roughness value of 0.088 (σ=0.011). The higher value indicates a rougher surface, thus there was an increase in overall roughness (Figure 8).

4.5 Mode of Failure

Samples were examined under a light microscope with 10X power to determine the nature of flexural failure. An adhesive failure was classified as a failure between materials, and no repair resin was left on the native bonding interface. A mixed failure showed some residual repair resin left on the native resin bonding interface. Initial failures for control samples were 42% adhesive (n=5) and 58% mixed failures (n=7).
Samples with 3 days of exposure to *Candida* biofilm had adhesive failures of 58% (n=7) and 42% (n=5) mixed failures. For 12 days of exposure to *Candida* biofilm, adhesive failures were 75% (n=9) and 25% mixed (n=3). After failure (Figure 9), a general trend towards an increase in adhesive versus mixed failures is seen as exposure interval increased. Scanning electron microscopy images were taken of both adhesive and mixed failures to confirm the failure mode (Figure 10).
5. Discussion

5.1 Discussion

This study investigated the effects of biofilm on the mechanical properties of denture acrylic and repair strength. The hypothesis that colonization by *C. albicans* biofilm would negatively affect mechanical properties was accepted for repair flexural strength (p<0.05) and microhardness (p<0.05). The hypothesis was rejected for surface roughness and flexural strength on unrepaired samples. Fracture strength and microhardness were statistically significantly affected by the presence of *Candida* biofilm and showed an unfavorable change in their properties after exposure to the biofilm.

After exposure to biofilm, the mechanical properties of the denture acrylic and repair were negatively affected to a certain degree, some statistically significant. It may be speculated that the presence of biofilm may affect and alter the surfaces associated with denture acrylic repair. This possible surface alteration may inhibit bonding between denture repair resin and native denture acrylic. This possible lack of chemical bonding among repair material and denture base could be attributed to the reason the repair strength of denture acrylic after exposure to *Candida* biofilm decreased.

The prevalence of *Candida* species on the removable prosthesis of edentulous patients is well documented in the literature.(7–9,35–37) Oral biofilm has been found to form on acrylic prosthesis after only a few hours of microbial exposure *in vitro*. (5) The continual accumulation of biofilm poses many mechanical and biological problems for
the prosthetically assisted patient. The buildup on biofilm on acrylic prosthesis is not helped as patients have been found to not adequately cleanse their prosthesis at home. This may not be only due to patient’s shortcomings, as many patients report they never even receive proper hygiene instructions. (45)

Repair strengths of acrylic prosthesis are documented to be between 25% and 80% of original strength. The most common repair method is chairside autopolymerizing resin. This method is popular because it is low cost and can be completed in one visit. However, this method is not without drawbacks, as it is also the weakest of all repair methods. In this study, autopolymerizing resin was used for repair to provide the most clinically relevant data. (46–48) Acrylic samples that underwent repair after exposure to C. albicans biofilm showed flexural failure strengths that were 75% less than unrepaired control samples.

An explanation for why the flexural strength of acrylic samples decreased through the study is the bonding surfaces may have been contaminated after the exposure to C. albicans biofilm. In normal clinical practice when a denture complication is encountered, the bonding surface of denture acrylic may be treated by removal of the surface layer, air particle abrasion, or application of a bonding agent before repair. The clinical goal is to remove any residual defects on the acrylic and provide ample space for repair material. In this study, the goal was to evaluate the effect of biofilm from Candida, so the only treatment to samples was disinfection by Cavicide®. No mechanical preparation or alteration of the acrylic surface to be contacting the repair resin was performed, which
differs from normal clinical practice. An increase in the amount of biofilm present may affect the bond formed between repair resin and denture acrylic. Examination of the failure method after flexural strength testing showed an increase in cohesive failures as the time interval of Candida exposure increased. (Figures 9,10) The acrylic samples that had been exposed to C. albicans biofilm for 12 days showed almost all cohesive failures, which may possibly be interpreted as a complete failure of any bond formation.

Fracture of denture material is due to concentrations of stress and strain within the acrylic, eventually leading to failure. (15,16) Flexural strength tests show the high elasticity and resistance to deformation of denture acrylic (Figure 3). This makes denture acrylic suitable to mastication, as it can withstand the forces placed on it during function. Over the lifetime of the prosthesis, these forces will remain as residual stresses with the acrylic. This can eventually lead to failure of the acrylic. Acrylic fractures are common, (2,18,19) so repair is mandatory. A patients masticatory force is increased by use of implants to support or retain prosthesis (3), however implant prosthesis are not without complication. With the increase in occlusal forces capable by implant supported or retained prosthesis (4), this stresses concentrated with the acrylic may lead to an increase in acrylic fracture.

Our study did not show a significant difference in flexural strength in unrepaired acrylic samples and control samples after biofilm exposure. Sahin and colleagues (25) found that exposure to various microorganisms affected the mechanical strength of denture acrylic by three-point bend, even without altering the samples for repair. The
three-point bend test and sample dimensions used by Sahin and colleagues were different than the ones used in this study. This may explain the difference in outcome of flexural strengths between the studies. Sahin and colleagues concluded that microorganisms quickly colonize over the surfaces of denture acrylics and may be present over a majority of the acrylic surfaces after a short time. The drop in mechanical properties may be due to the decomposition of the acrylic by biofilm formation and/or biocorrosive activity of microorganisms. A similar conclusion is proposed after this study, and that the bonding interface was broken down and negatively affected by denture biofilm, leading to a decrease in repair strength.

*Candida* metabolism releases an acidic by product, and this may attribute to the negative effects on the mechanical properties of denture acrylic observed in this study.(10,23–25) A decrease in microhardness means the surface of the acrylic became softer after *Candida* exposure. One possible explanation for the decrease in hardness could be a degradation of the surface after exposure to biofilm. This degradation could be due to metabolism by *Candida albicans*. Metabolism by *Candida albicans* is known to release acid as a by-product. This acid may lead to a biocorrosive activity, causing a breakdown of the surface and subsequently decreasing the microhardness.(25)

The frequency of *C. albicans* on acrylic prosthesis has been shown many times, most often resulting in a stomatitis type reaction in the denture wearer.(7,9,36,38,40,41,51) With this known knowledge, clinicians must recognize the presence of *Candida*, and determine ways to limit its prevalence. Current clinical
practice involves soft denture liners, hygiene instructions, and medications to help relieve symptoms from *Candida* in patients. Other methods are currently being researched as ways to help limit and control *Candida*.

Adherence of *Candida albicans* can be influenced by the surface roughness and porosity *in vitro*. (52,53) Human saliva has been found to prevent adherence of *Candida* to the surface of denture acrylics, while an increased porosity and roughness may increase adherence. There is a balance that must be found through *in vivo* testing. As the roughness of an acrylic increases with aging, this may promote further colonization and biofilm formation.

Acrylics are porous, and this porosity can have many detrimental effects to the mechanical properties. (54) Porosity can result in staining, adherence of substances, and also cause high internal stresses in the material, leading to vulnerability and distortion. *Candida albicans* may be able to penetrate into these pores, causing negative effects on the mechanical properties. When repairing denture acrylics, the depth of biofilm penetration must be understood. This may help the clinician in deciding when a prosthesis may be repaired or if the pores are saturated and replacement is necessary.

Ethylene oxide sterilization is the preferred method of sterilization for denture acrylics as acrylic is heat sensitive, and conventional autoclaving would result in extensive distortion and damage to the samples. Previous research has examined that ethylene oxide can affect polymers by esterifying bonds, resulting in altered physical
properties. Ethylene oxide was the only viable available sterilization option available for this study for denture acrylic. Other research has shown that although ethylene oxide sterilization may affect mechanical properties, it does not change the efficacy of the material for medical application.

Measurements errors are present in several areas of this study, and work was done to limit them as much as possible. Samples were treated identically in order to avoid extra induced error. Samples were fabricated in several groups due to limits in processing capabilities, but samples were randomly assigned to groups to ensure equality of groups. The monomer and polymer came from the same bottle for all groups to ensure consistency of the material.

Sample polishing was completed to 800 grit for all samples except the surface roughness, which was completed to 1200 grit. This was performed after pilot testing showed difficulty of the machine in reading sample roughness. This is not representative of the intaglio surface of a denture seen clinically, as the surface is much more polished than normal clinical prosthesis. This may have led to less attachment and adhesion by Candida, as previous research has reported attachment and colonization by Candida is best tested on a slightly roughened surface. However, polishing standardized all samples so that testing conditions would be as uniform as possible.

A 3-point bend test was selected as this was used in several previous research studies. This test is indicated ADA Spec 12 for denture polymers for determining
A four-point bend test would have been applicable for testing a broad area, however this study attempted to determine failure at a small bonding interface, thus a three-point bending test was selected.

The load to failure was reported instead of strength which was done for several reasons. The sample size and testing parameters were very carefully controlled and all samples were the same size to ± 0.1mm. Reporting of materials can occur by one of two methods: strength or load at failure. Strength is a function of stress, and reporting is based on removing errors due to variable in specimen dimension. In this study, the load of failure was reported due to the control of sample size to ± 0.1mm.

Two common microhardness tests are available for materials research. A Vickers microhardness test and Knoop microhardness test. The Vickers test uses a pyramidal diamond to indent a surface in an attempt to determine a materials resistance to plastic deformation. It is the most universal microhardness test, and is best for very hard materials such as metals. A Knoop microhardness test uses a pyramidal diamond and is best for brittle or thin materials. Due to acrylics thin nature in this study, and the availability of testing apparatus, a Knoop microhardness test was used. The range of data can be seen in appendix 1. This large range of data results in a need to limit sampling error. This was achieved by taking 5 measurements per sample and obtaining an average to calculate the Knoop microhardness for each sample. The inherent surface irregularities and porosities present in denture acrylic result in the wide range of values through the sample. Five measurements were taken per sample as this is what were
permitted by the sample dimensions. This ensured that data points were collected far enough apart that deformation from testing would not influence adjacent data points. The overall means of the samples then must be compared, so in essence 60 data points were taken for each time interval and averaged. This helped limit error as to as large of extent as possible.

Several previous studies have evaluated the incorporation of antimicrobial agents into denture liners or denture acrylics to help prevent *Candida* proliferation and biofilm formation.\(^\text{42–44}\) *In vitro* studies have shown some success, however *in vivo* application and proven clinical outcomes do not yet exist. An issue with incorporation with metals such as silver, magnesium, or zinc is the relative dose and possible toxicity to the patient. Soft lining denture materials are also prevalent in *Candida* colonization.\(^\text{41,59}\) To ensure optimal health to the patient, liners must be replaced frequently to avoid denture stomatitis.\(^\text{39–41}\)

The decrease in repair strength and degradation of mechanical properties is in line with results by Rinastiti and colleagues research on oral biofilm and its effect on repair strength of dental composites.\(^\text{10}\) The negative effect of *C. albicans* biofilm on the mechanical properties of denture acrylics is similar to the oral biofilm model related to dental composites.
5.2 Clinical Implications

*Candida albicans* is frequently associated with acrylic based prosthesis. The presence of this fungus may have a negative effect on the mechanical properties and repair strength of denture acrylics when complications are encountered. The clinician must be aware of the presence of *Candida albicans* when preparing to repair an acrylic prosthesis. A decision must be made whether repair versus recommending replacement of the prosthesis.
5.3 Limitations of the study and future research:

Several limitations are present at the conclusion of this study. This was an *in vitro* study, thus clinical studies must be performed in order to determine the exact clinical relevance of the data. After clinical studies, more definitive recommendations can be made to the clinician to ensure proper treatment of denture acrylic when a repair is needed.

Thermocycling was not performed in this study due to lack of available armamentarium at the time the research was conducted. This may slightly alter values when comparing to *in vivo* findings.

This study was limited to *C. albicans* and subsequent biofilm formation. In further research, a multi species oral biofilm could be used to more closely replicate what is present *in vivo*. With this, the amount of biofilm grown on samples could be performed by determining the mass of biofilm produced. Confocal studies could also be done to determine the thickness of biofilm present, and the depth of penetration. Acrylic is a porous material, and in order to make clinical recommendations as to the extent of acrylic remove necessary in order to proper eliminate oral biofilm.

Future research can now be performed to expand on various microbes effect on denture acrylic mechanical properties and repair. A mixed species biofilm model could be used to more adequately represent conditions encountered *in vivo*. Other possibilities
for species to be studied include *Streptococcus oralis, Streptococcus sobrinus, Actinomyces naeslundii, Fusobacterium nucleatum, Veillonella parvula, Streptococcus mutans, Escherichia coli, Streptococcus aureus,* or *Pseudomonas aeruginosa.* All these microbes occur frequently in the oral cavity, and their colonization could be studied for effect on mechanical properties of acrylcs. In this study, no preparation of the bonding interface was done, which varies from normal clinical practice when a complication is found. Further research into methods of manipulation of the affected interface can be done to ensure ideal conditions are prepared to create the most optimal denture repair.

Saliva could have some effect on the adhesion of microbes to denture acrylic. In future research, samples could be coated with artificial saliva to more closely replicate clinical conditions.
6. Conclusions

Within the limitations of the study, the following conclusions were drawn:

1. The exposure of denture acrylic to *Candida albicans* biofilm had a negative effect on the repair strength of denture acrylic. Significant differences (p<0.05) were reported for both the 3 days and 12 days of biofilm formation. The repair strength after 12 days was less than 3 days, and longer exposure intervals led to a further decrease in repair strength.

2. The exposure of denture acrylic to *Candida albicans* biofilm did not affect the fracture strength through a three-point bend test of unrepair ed denture acrylic. Fracture strengths before and after exposure to *Candida albicans* and subsequent biofilm formation were unchanged.

3. The exposure of denture acrylic to *Candida albicans* biofilm had a negative effect on the microhardness of denture acrylic. Significant differences (p<0.05) were reported for both 3 and 12 days of biofilm formation. Exposure to *Candida albicans* and subsequent biofilm formation made the surfaces of denture acrylic softer.

4. The exposure of denture acrylic to *Candida albicans* biofilm did not have a statistically significant effect on the surface roughness of denture acrylic (p>0.05). The surface roughness did gradually increase over time with exposure to *Candida albicans* biofilm, and surfaces were rougher, but not to a statistically significant level.
5. As the exposure time to *Candida albicans* biofilm increased, there was an increasing trend to more adhesive failures at the bonding interface in the repaired samples and less mixed failures.
Figure 1: Flask of standardized jigs for fabrication of acrylic samples

Figure 2: Completed denture acrylic samples
Figure 3: Three-point bend test to determine flexural strength

Figure 4: SEM of *Candida albicans* biofilm on denture acrylic after A) 3 days B) 12 days
Figure 5: Flexural Strength by 3-point bend of failure of repaired and non-repaired samples after different time periods of *Candida albicans* biofilm growth and subsequent sample repair.
Figure 6: Microhardness of acrylic samples before and after *Candida albicans* biofilm formation for 3 and 12 days.
<table>
<thead>
<tr>
<th>C. albicans exposure time (days)</th>
<th>Initial</th>
<th>Final</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 days</td>
<td>0.060</td>
<td>0.865</td>
<td>0.084</td>
</tr>
<tr>
<td>12 days</td>
<td>0.068</td>
<td>0.088</td>
<td>0.054</td>
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</tbody>
</table>

Figure 7: Table of initial and final surface roughness values after denture acrylic exposure to *Candida albicans* biofilm formation for 3 and 12 days

Figure 8: Surface roughness profile of denture acrylic samples exposed to *Candida albicans* biofilm for A) 3 days B) 12 days
<table>
<thead>
<tr>
<th>Days</th>
<th>Adhesive (%)</th>
<th>Mixed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5 (42)</td>
<td>7 (58)</td>
</tr>
<tr>
<td>3</td>
<td>7 (58)</td>
<td>5 (42)</td>
</tr>
<tr>
<td>12</td>
<td>9 (75)</td>
<td>3 (25)</td>
</tr>
</tbody>
</table>

Figure 9: Table of classification of failure of bonding interface between native acrylic and repair resin

Figure 10: SEM of denture acrylic repaired interfaces after failure by 3-point bend test A) Adhesive failure  B) Mixed failure
### 7. APPENDICIES

<table>
<thead>
<tr>
<th>Days</th>
<th>Repaired N (σ)</th>
<th>Non-Repaired (σ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.4N (6.57)</td>
<td>46.2N (9.2)</td>
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<tr>
<td>3</td>
<td>5.7N (2.81)</td>
<td>47.2N (5.84)</td>
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<tr>
<td>12</td>
<td>3.6N (2.5)</td>
<td>44.5N (7.56N)</td>
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Appendix A: Descriptive statistics for flexural strength

<table>
<thead>
<tr>
<th>Days</th>
<th>Initial Microhardness (σ)</th>
<th>Final Microhardness (σ)</th>
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</thead>
<tbody>
<tr>
<td>3</td>
<td>388.3 (31.7)</td>
<td>364.5 (16.6)</td>
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<tr>
<td>12</td>
<td>402.4 (42.3)</td>
<td>362.2 (17.6)</td>
</tr>
</tbody>
</table>

Appendix B: Descriptive statistics of knoop microhardness
REFERENCES


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- American Academy of Fixed Prosthodontics, February 2014
- University of Illinois at Chicago College of Dentistry, March 2014