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IN VITRO ANALYSIS OF ATTACHMENT OF *CANDIDA ALBICANS* TO DENTURE BASE ACRYLIC RESINS FABRICATED BY THREE DIFFERENT METHODS

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ABSTRACT

IN VITRO ANALYSIS OF ATTACHMENT OF *CANDIDA ALBICANS* TO DENTURE BASE ACRYLIC RESIN FABRICATED BY THREE DIFFERENT METHODS

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PROSTHODONTICS, 2020

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Introduction: Advancement in CAD/CAM processes, digital dentistry, and acrylic resin materials has provided time-efficient and cost-effective ways to fabricate complete dentures. 3D-printed dental resins, and their unique processing techniques, are relatively new in concept to dentistry. Biocompatibility properties of printed resins, specifically pathological microbial adhesion to their surfaces, are not well understood. The purpose of this study was to compare the biofilm forming capacity of *Candida albicans* to denture resin surfaces fabricated by conventional heat processing, CAD/CAM milled, and 3D-printed manufacturing methods.

Materials and Methods: 48 standardized samples (10mm x 10mm x 2mm) were fabricated and divided into four groups: 12 conventional (Lucitone 199; Dentsply Intl.), 12 CAD/CAM milled (AvaDent CAD/CAM Denture Base Puck; Global Dental Science), 12 3D-printed with FormLabs (Dentca 3D denture base; Dentca, Inc.), and 12 Lucitone Digital Print™ 3D Denture Resin (Dentsply Intl.). *C. albicans* isolates were grown and diluted to 1×10^7/mL concentration in Sabouraud Dextrose Broth. *C. albicans* were allowed to attach to the samples for 24 hours at 37°C, and washed with phosphate-buffered saline (PBS) to remove non-adherent cells. The adherent cells were plated on

Sabouraud Dextrose Agar in duplicates and Colony-Forming unit (CFU) counts were quantified.

Results: A one-way analysis of variance (ANOVA) analysis among *C. albicans* CFUs on the four denture acrylic resin groups showed that there were no statistically significant differences in the mean attachment capacity of *C. albicans* to these acrylic resin types ($p = 0.151$).

Discussion: Based on the statistical analysis, the result suggests no significant differences were seen in adhesion and formation of *C. albicans* regardless of the manufacture technique of each denture base polymers tested. The data suggest that normality was not satisfied due to high variability of each group tested. The hypotheses of this study that CAD/CAM-milled and 3D-printed denture base acrylic resins will demonstrate lower adhesion and formation of C. *albicans* on the denture base surfaces compared to conventional PMMA denture fabrication process was rejected.

Conclusions: Within the limitations of this study, CAD/CAM-milled and 3D-printed denture resin surfaces perform as well as conventional "gold standard" resin surfaces in preventing *C. albicans* biofilm attachment. Further studies are recommended to include: larger sample size to allow elimination of outliers, thermocycling to simulate aging samples to understand longevity, incorporate SEM imaging to evaluate surface roughness of samples, and validate adherence results using a cell proliferation assay kit (e.g., XTT). This study and future studies are important as 3D manufacturing has broad applications and may transform dentistry.

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LIST OF ABBREVIATIONS

Abbreviations

1. CAD/CAM: computer-aided design and computer-aided manufacturing
2. PMMA: polymethyl-methacrylate
3. *C. albicans: Candida albicans*
4. PBS: phosphate-buffered saline
5. SDB: Sabouraud dextrose broth
6. SDA: Sabouraud dextrose agar
7. ATTC: American Type Culture Collection
8. ANSI: American National Standard Institute
9. ADA: American Dental Association
10. ISO: International Organization for Standardization
11. XTT: XML Tunneling Technology
12. ANOVA: A One-way Analysis of Variance
13. CFU: Colony-Forming Units
14. SD: Standard Deviations
15. SEM: Scanning Electron Microscopy

CHAPTER I: REVIEW OF THE LITERATURE

HISTORY

Conventional complete denture bases can be fabricated using a number of materials and methods. History of denture fabrication dates back to 700 B.C., made out of wood, bone, and ivory. Porcelain dentures were introduced by a French dentist in the late 1700s, and one of the first gold denture bases was made for U.S. President George Washington in 1794 by Dr. John Greenwood. [1] In 1839, Charles Goodyear discovered vulcanized rubber, which was then introduced as a denture base material in 1853. [2] Despite the unaesthetic and unhygienic properties of vulcanite denture bases, there were no other base materials until 1936 with an introduction of polymethyl-methacrylate (PMMA), as a heat-processed material. [1] By the mid-1940s, approximately 95% of denture bases were fabricated with PMMA resin. [2] Since then, many efforts were made to enhance the properties of PMMA. There are various methods for complete denture base fabrication which include heat, chemical, light, and microwave energy. The latest methods include milling (subtractive manufacturing) and 3D-printing (additive manufacturing) utilizing computer-aided design and computer-aided manufacturing (CAD/CAM).

DENTURE BASE MATERIALS

According to the American National Standard Institute (ANSI) and American Dental Association (ADA) classification (ISO 20795-1:2013), denture base polymers are categorized into different types and classes (Fig.1). Based on its class and type, processing technique is also unique with inherent advantages and disadvantages. For this study, three denture base materials were tested. The first material was a heat-polymerized PMMA (type I) in a powder-liquid system (class I), conventionally processed by heat and compression (Lucitone 199; Dentsply Intl). The second material was a pre-polymerized PMMA denture base resin puck (AvaDent; Global Dental Science), which is processed by injection under high temperature and pressure. The resin pucks are milled with aid of CAD/CAM technology. The last materials were 3D-printed denture base resins (Dentca Denture Base; Dentca, Inc. and Lucitone Digital Print^{TM} 3D Denture Resin; Dentsply Intl) that are photosensitive urethane methacrylate-based resins, also manufactured by aid of CAD/CAM technology.

4 Classification

Denture base polymers covered by this part of ISO 20795 are categorized into the following types and classes:

- Type 1: Heat-polymerizable materials
  - Class 1: Powder and liquid
  - Class 2: Plastic cake
- Type 2: Autopolymerizable Materials
  - Class 1: Powder and liquid
  - Class 2: Powder and liquid for pour-type resins
- Type 3: Thermoplastic blank or powder
- Type 4: Light-activated materials
- Type 5: Microwave cured materials

Figure 1. ANSI and ADA classification ISO 20795-1:2013

Lucitone 199 Denture Base Resin (Dentsply Intl):

The conventionally processed denture base used in this study was a heat processed PMMA resin system, which includes powder and liquid components. The liquid contains non-polymerized methyl methacrylate and hydroquinone as an inhibitor to prevent polymerization of the liquid during storage. The powder contains pre-polymerized

PMMA resin, in the form of small beads, and a small amount of benzoyl peroxide as an initiator. The two components are mixed together and heat is applied. This initiates polymerization by decomposing benzoyl peroxide to produce free radicals. The polymerization cycle is completed in a controlled temperature water bath via a compression molding technique. [3]

AvaDent CAD/CAM Denture Base Puck (AvaDent; Global Dental Science):

The pre-polymerized PMMA pucks used for milling denture bases are made by an injection molding technique under high pressure and temperature. [4] In recent literature, several authors stated the pre-polymerized PMMA pucks have demonstrated enhanced physical properties such as minimal polymerization shrinkage, [4,5,6] less porosity, and less bacterial adherence. [7,8]

Dentca 3D Denture Base (Dentca, Inc.):

Dentca 3D denture base is composed of urethane methacrylate-based photopolymerized resins. [9] It is mainly utilized for fabrication of interim prostheses, or trial dentures, and is rarely used definitively. [10] However, after recent FDA approval, it is now classified as a Class II long-term biocompatible denture base resin. Mechanical properties of Dentca resin are not known at this time. A FormLabs Form 2 desktop stereolithography 3D-printer was used in this study.

Lucitone Digital Print™ 3D Denture Resin (Dentsply Intl):

Lucitone Digital Print™ 3D Denture Resin is composed of urethane methacrylate photosensitive polymer liquid resins. The liquid resin is polymerized by an ultraviolet light, layer by layer, utilizing Carbon M-Series printer workflow. Lucitone Digital Print™ recently became available to U.S. dental laboratories (September 2019) and no publications are yet available for this material.

SIGNIFICANCE AND DEMAND FOR COMPLETE DENTURES

Despite an anticipated decrease in the age-specific rate of edentulism, the demand for complete dentures will continue to increase as the size and age of the older population increases. [11] According to the 2011-2012 National Health and Nutrition Examination Survey in the U.S., the prevalence of edentulism was 13% in patients between the ages of 65-74 years old and 26% in patients older than 75 years old. [12] For the 75 years and older group, their economic status did not play a significant role in the occurrence of edentulism. [13] With an anticipated increase in elderly population and corresponding denture patients, there is greater interest in digitally fabricated complete dentures. To date, analyses of digital CAD/CAM denture have focused on manufacturing techniques, retention, stability, esthetics, and masticatory functions. However, biocompatibility and physical properties, which include microbial adhesion to denture surfaces manufactured by CAD/CAM methods, are not yet known.

COMPUTER-AIDED DESIGN AND COMPUTER-AIDED MANUFACTURING (CAD/CAM) TECHNOLOGY

CAD/CAM technology has been utilized by automotive and aerospace manufacturers since the 1960s. In the early 1980s, the technology was adapted in dentistry to fabricate single-unit restorations. [14] Since then, CAD/CAM technology has been expanded to many other clinical and laboratory procedures. However, the complexity of clinical techniques in denture fabrication has prevented CAD/CAM adaptation in denture manufacturing. [14] With recent technical advancements and flexibility of combining digital and conventional workflows, the interest in CAD/CAM digital workflow of complete dentures in both clinical and laboratory practices has increased significantly. [6,14,15,16] CAD/CAM technology in denture fabrication workflow could decrease the number of patient visits and clinical treatment time by 50%. [6,15,16,17,18,19] A systematic review published in 2013 by Bidra and colleagues [19] summarized disadvantages of conventional denture fabrication as follows: (1) a minimum of four to five patient visits and post-delivery follow ups, (2) high treatment cost due to extensive chair-time, (3) laboratory expenses, (4) denture material limitations, and (5) additional chair and lab time and cost to duplicate/replace dentures. Bidra and colleagues highlighted advantages of CAD/CAM dentures to include (1) reduced number of patient visits, (2) enhanced properties of the denture material and better fit, (3) reduced costs for both patients and clinicians, (4) easily reproducible, (5) improvement in standardization of clinical research, and (6) better-quality control. Generally, CAD/CAM dentures require two to three clinical appointments, depending on the preferred method of obtaining clinical records, and need for a trial denture prior to final prosthesis delivery. [15]

There are two methods to fabricate CAD/CAM dentures. The first method, more frequently used, is a subtractive process of milling the denture base from a pre-polymerized resin puck as described previously. [4] The highly condensed pre-polymerized material leads to less residual monomer and polymerization shrinkage, has fewer micro-porosities, and superior physical/mechanical properties. [4,6,16] The second method is by 3D-printing, which utilizes an additive (rapid prototyping) manufacturing technology. There are two commercial manufacturers of 3D-printed denture bases in U.S.: Dentca 3D Denture Base (Dentca, Inc.) and Lucitone Digital Print™ 3D Denture Resin (Dentsply Intl). Both materials use photosensitive liquid resins which are polymerized by an ultraviolet light or a visible light source, layer by layer on a supporting structure. [10] Compared to traditional compression molding and heat processing, CAD/CAM-milling and 3D-printing technologies are relatively new and demonstrate potential clinical benefits. [19] However, quantification of microbial adhesion to materials used in these three different fabrication methods is not currently known. Pathogenic microbial adhesion can increase the risk of denture related stomatitis in older patients.

ORAL CANDIDIASIS

Oral *candidiasis,* in the form of *candida*-associated denture stomatitis, is a common disorder and most often asymptomatic to denture wearers. [20,21] It is characterized as an inflammatory reaction of the oral mucosa covered by the dental prosthesis. Most patients do not experience symptoms such as pain, itching, or burning sensations. [20,21] However, when symptomatic, the patient may display mucosal bleeding and swelling, and may experience burning and pain, halitosis, unpleasant taste, and xerostomia. [22] Denture stomatitis is primarily diagnosed by presence of inflammation and erythema on the palatal mucosa of complete and partial denture wearers. There are three classifications to denture stomatitis: type I refers to an initial stage of localized inflammation involving redness and hyperemia of minor salivary glands; type II is more common with diffused reddening of the entire denture bearing area, generalized hyperemic, smooth, and atrophic mucosa; type III (also known as a granular-type) is considered due to untreated type II, with hyperemic and papillary mucosa. [22,23] The incidence of denture stomatitis varies. A review by Gendreau and Loewy found that prevalence ranges from 15% to 71%. [21] The etiology of denture stomatitis is multifactorial. It has a number of associative factors, rather than a single cause, such as poor hygiene, pathogenic *Candida* infection, or continual wear of dentures. [21]

Among the many predisposing local factors, the denture surface itself may serve as a reservoir of microbial plaque accumulation. *Candida* adhesion to the denture resin plays a critical role in the pathogenesis of denture stomatitis. [21,23,24,25,26] *Candida albicans* (*C. albicans*), along with other species, are found naturally in the oral cavity. However, *C. albicans* is the predominant and major causative microbe for denture stomatitis. [21,22,23,24,25,26] Webb and colleagues [27] showed that the mechanism of *C. albicans* attachment could be due to hydrophobic and electrostatic forces between the microorganisms and the denture base. Denture stomatitis is mainly associated with *C. albicans*, along with other oral environment factors such as saliva, pH, bacteria, and formation of fungal hyphae. Ramage and colleagues [28] found biomaterial properties such as surface roughness and chemical properties may also affect the initial attachment of

Candida. The initial attachment of *Candida* is followed by cell division, proliferation, and biofilm formation. [28] Once the biofilm is established, it is more tolerant to antifungal therapy and host immune response. [28]

Physicochemical properties of denture resin materials, and the manner in which they are processed, may influence the ability of oral microbes to colonize, form biofilms, and penetrate denture surfaces. Surface characteristics of acrylic resins such as roughness, hardness, and wettability have been reported as causative factors in predisposition to higher susceptibility for denture stomatitis. [29] Al-Dwairi and colleagues [29] recently reported that milled CAD/CAM PMMA denture bases exhibited superior surface properties compared to conventional heat-polymerized denture bases. Kalberer and colleagues [10] compared the trueness of CAD/CAM milled and rapidly prototyped (3D-printed) complete dentures and found both techniques demonstrated clinically acceptable levels of precision when compared to the conventional method. The authors, however, did not address mechanical properties, biocompatibility, material and color stability, and residual monomer levels of the printed denture base materials.

PURPOSE OF THE STUDY

The purpose of this study was to compare the attachment capacity of *C. albicans* to denture acrylic resins fabricated by conventional compression molding, CAD/CAM-milling, and 3D-printed manufacturing methods.

HYPOTHESIS

CAD/CAM-milled and 3D-printed denture base acrylic resins will demonstrate lower adhesion and formation of C. *albicans* on the material surfaces compared to samples made by the conventional PMMA fabrication process.

CHAPTER II: MATERIALS AND METHODS

Four commercially available denture base materials were evaluated in this *in-vitro* study (Table 1). All samples were prepared according to the manufacturers' instructions.

Table 1. Denture base polymers tested

Product Name	Polymer Type	Method of Fabrication	Manufacture
Lucitone 199	PMMA- High impact	Heat and Compression	Dentsply Intl
AvaDent CAD/CAM Denture Base Puck	PMMA-Pre-polymerized	Five-axis milling	Global Dental Science
Dentca 3D Denture Base	Methacrylate-based photopolymer liquid resin	3D-printed	Dentca, Inc.
Lucitone Digital Print™ 3D Denture Resin	Urethane methacrylate photosensitive polymer liquid resin	3D-printed	Dentsply Intl

DENTURE BASE SAMPLE PREPARATION:

The study samples were designed using 2D CAD Drafting and 3D Design by SOLIDWORKDS® computer software. The design had dimensions of 87mm (l) x 10mm (w) x 2mm (d), with 1mm cut lines spaced every 10mm (fig. 2). The 1mm cut lines allowed sectioning with minimal effort to obtain uniform final samples. The file of the design was formatted to the Standard Tessellation Language (STL) for milling or printing. A total of forty-eight samples, measuring 10mm (1) x 10mm (w) x 2mm (h), were prepared from four commercially available denture base materials according to the manufacturers' instructions.

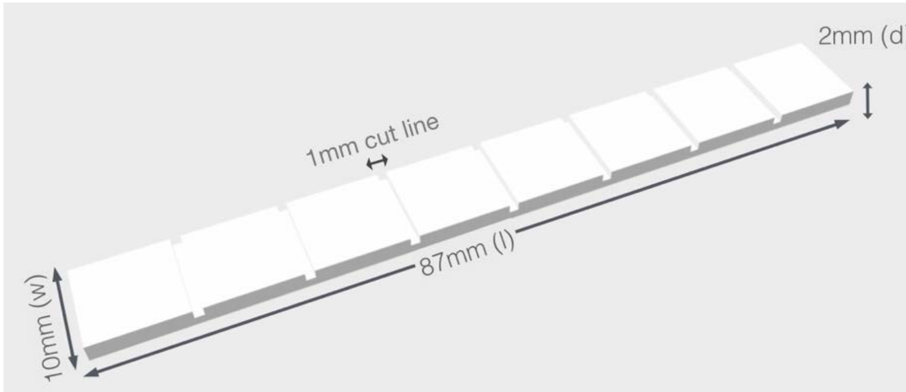


Figure 2. Standardized sample dimensions 87mm (l) x 10mm (w) x 2mm (d)

The heat-polymerized denture base resin (Lucitone 199; Dentsply Int) was processed using a conventional flasking and pressure-pack technique. A pattern of the sample was milled in wax (ProArt Wax Disc for Zenotec; Wieland Dental+Technik GmbH & Co. KG) and used for conventional processing. The pattern was flasked and eliminated from the stone mold. The resin was mixed, packed, and cured in a water bath at 73°C ±1℃ for 9 hours, followed by 30 minutes at 100°C, as recommended by the manufacturer. After deflasking, excess flash was removed with carbide burs (E-cutter Kit 1108.11 BrasselerUSA). Light polishing was done with silicone resin base acrylic polishing burs (BrasselerUSA). All resin samples were washed and stored in distilled water to minimize influence of residual monomers or toxic constituents on cell viability. They were subsequently sterilized under an ultraviolet light unit for 10 minutes.

The CAD/CAM-milled denture base samples were prepared: the STL design file was nested, and the tool path calculated, in Zenotec software (Wieland Dental+Technik GmbH & Co. KG). AvaDent CAD/CAM samples were milled from the corresponding material puck. After milling, samples were sectioned along the 1mm cut lines. Excess material and rough edges were polished with a silicone resin base acrylic polishing bur (BrasselerUSA). All resin samples were washed and stored in distilled water, and sterilized under an ultraviolet light unit for 10 minutes.

The CAD-CAM printed resin material samples were prepared: the STL file was sent to two respective dental laboratories with instructions to print the samples. Dentca 3D denture base samples were requested through the Walter Reed National Military Medical Center 3D Medical Application Center. The Dentca 3D denture base samples were printed following FormLabs Form 2 desktop stereolithography 3D printer protocol. Lucitone Digital Print™ 3D denture resin samples were requested through Absolute Dental Services in Durham, NC. The Lucitone Digital Print™ 3D denture resin samples were printed following the Carbon M-Series printer workflow. All printed samples were returned fully cured and processed, without sectioning or polishing. All samples were sectioned along the 1mm cut lines and dimensions were verified. Excess material and rough edges were polished with a silicone resin base acrylic polishing bur (BrasselerUSA). All resin samples were washed and stored in distilled water, and sterilized under an ultraviolet light unit for 10 minutes.

Note: High-polishing of samples was intentionally omitted in order to mimic typical denture intaglio surfaces.

MICROORGANISM SAMPLE PREPARATION:

C. albicans (American Type Culture Collection 90028) was cultured in Sabouraud dextrose broth (SDB) for 24 hours at 37°C. The cells were harvested and washed twice with phosphate-buffered saline (PBS). The cells were diluted to $1x10^7$/mL in SDB. The denture resin samples were placed in 24-well plates containing 2 ml of the inoculum and incubated at 37°C for 24 hours. Following incubation, the resin samples were transferred to new 24-well plates and the samples were washed with sterile PBS buffer to remove non-adherent cells. The adherent cells were collected from the resin samples by vortexing for 2 minutes at 1000 rpm (Fisherbrand™ Digital MultiTube Vortexers). The adherent cells were plated on Sabouraud dextrose agar (SDA) plates in duplicates. Colony-forming unit (CFU) counts were determined after 24hour incubation at 37°C.

CHAPTER III: STATISTICAL ANALYSIS AND RESULTS

STATISTICAL ANALYSIS

Sample size was calculated with assumption of a clinically significant effect size between each group tested, and from results of previously published studies. Controlling for the probability of committing a type I error at 5% ($\alpha = 0.05$) and a power of 80%, 12 acrylic resin samples per test group was recommended. Four groups, composed of 12 samples per group, were analyzed. A one-way analysis of variance (ANOVA) statistical method was used to compare *C. albicans* isolate adhesion values for the conventional, CAD/CAM-milled and 3D-printed denture bases. A significance level of <0.05 was considered statistically significant.

RESULTS

The mean *C. albicans* isolate adhesion values (CFU/mL) and standard deviations (SD) for the conventional, CAD/CAM-milled, and 3D-printed denture bases are presented in Table 2.

Table 2. Means and standard deviations of CFU/mL of *C. albicans* isolate adhesion for all groups evaluated. No statistically significant differences were observed among all groups ($P > 0.05$)

Product Name	**Mean (CFU/mL)**	**SD**
Lucitone 199	109.75	52.32
AvaDent CAD/CAM Denture Base Puck	100.92	62.80
Dentca 3D Denture Base	81.70	47.17
Lucitone Digital Print™ 3D Denture Resin	65.58	34.81

Based on the statistical analysis, regardless of the manufacturing techniques of each denture base, no significant differences were seen in adhesion and formation of *C. albicans* (*p-value* = 0.015). Although the samples were prepared and cultured with high degree of reproducibility and with process standards, our data suggest that normality was not satisfied due to high variability of each group tested. This high variance could be due to biological cell-cycle/cell-division and different adherence capability of the *C. albicans* tested. When the mean CFU values of each test group were ranked, 3D-printed denture bases appear to have lower colony formation. However, due to a large SD and small sample size, it was determined that the differences in means were not significant. Therefore, the hypothesis of this study, that the surfaces of CAD/CAM-milled and 3D-printed denture base resins would demonstrate lower adhesion and formation of C. *albicans* compared to conventional PMMA material, was rejected.

CHAPTER IV: DISCUSSION

Surface characteristics of denture bases have significant impact on the degree of C. *albicans* adhesion and colonization. [30,31] Previous studies showed that CAD/CAM dentures (specifically milled materials), demonstrate superior mechanical and physical properties compared to conventional PMMA. [4,5,6,7,8,19] The results of this study, however, indicate that CAD/CAM materials are not superior in preventing *C. albicans* attachment compared to conventional PMMA. Other desired qualities such as esthetics, longevity, ease of maintenance, biocompatibility, etc. were not considered in this study.

Srinivasan and colleagues[32], showed that there were no significant differences in cell proliferation profiles between CAD/CAM-milled and conventionally fabricated denture bases. This observation can be explained, in part, by surface roughness created in the milling process. Although CAD/CAM PMMA resins demonstrate improved mechanical properties, milling instruments leave micro- and macro-surface imperfections. These imperfections create roughness in the final surface, and can lead to increased oral biofilm attachment.

Al-Fouzan and colleagues [8] assessed the adhesion of *C. albicans* to the surfaces of CAD/CAM and conventionally fabricated denture base materials. The authors performed surface roughness analysis using a non-contact optical three-dimensional profilometer. They concluded that conventional materials exhibited surface roughness that was significantly greater than CAD/CAM materials. [8] Following surface analysis, the authors also assessed four different *C. albicans* isolates, and their ability to adhere to the denture base groups. They found that regardless of strains, *C. albicans* adhered to the CAD/CAM denture resin with lower affinity compared to the conventional material. [8]

Recently, Murat and colleagues [33] compared the amount of adherent *C. albicans* to several CAD/CAM PMMA based polymers and conventional heat-polymerized PMMA after thermocycling (i.e. simulated aging). First, they evaluated surface roughness under scanning electron microscopy (SEM). They reported there were significant differences between conventional and CAD/CAM PMMA based polymers. [33] No differences were found among three CAD/CAM PMMA materials, however conventional PMMA polymers presented more porous and irregular surfaces. [33] The authors reported that long-term thermocycling may affect surface roughness by altering the surface energy/hydrophobicity of resin materials. [33] However, no clear relationship was seen between the hydrophobicity and direct influence on the adhesion of *C. albicans*. While a strong positive correlation was observed between *C. albicans* adhesion and the surface roughness of denture base polymers, the authors concluded that the CAD/CAM PMMA-based polymers may reduce *Candida*-associated denture stomatitis in long-term use. [33]

The results of this current study support Srinivasan and colleagues' findings. At this time, it is safe to state that CAD/CAM-milled PMMA material is similar to conventional PMMA material in *C. albicans* attachment, and not superior in prevention of biofilm development. To our best knowledge, this is the first study investigating microbial adhesion to 3D-printed denture resin surfaces. There are four published studies comparing differences between CAD/CAM-milling and 3D-printing in trueness or adaptation, however without biological observations. Yoon and colleagues [34] evaluated the trueness of printed, milled and conventional mandibular dentures. The authors concluded that the trueness of milled denture bases was significantly better than printed denture bases, however, no significant difference in tissue surface adaptation was observed between three different fabrication methods. [34] Kalberer and colleagues [10] evaluated the trueness of CAD/CAM-milled and 3D-printed denture bases, and concluded that milled bases were superior to printed in terms of trueness of the intaglio surfaces. [10] However, the authors stated that further studies are needed to address biomechanical and patient outcome measures to determine superiority of materials and techniques. [10] Lee and colleagues [35] found that overall accuracy was higher in CAD/CAM-milled and 3D-printed denture bases, when compared to injection molded bases. Hwang and colleagues [36] found that 3D-printed denture bases demonstrated better trueness and tissue surface adaptation, when compared to conventional and milled fabrication methods. These publications did not address the issues related to storage, repairing and relining, biocompatibility, and anti-microbial properties of 3D-printed denture acrylic reins. This current study, although not statistically significant, showed a general trend toward 3D-printed resin surfaces having less *C. albicans* colonies, compared to conventional and CAD/CAM-milled PMMA materials.

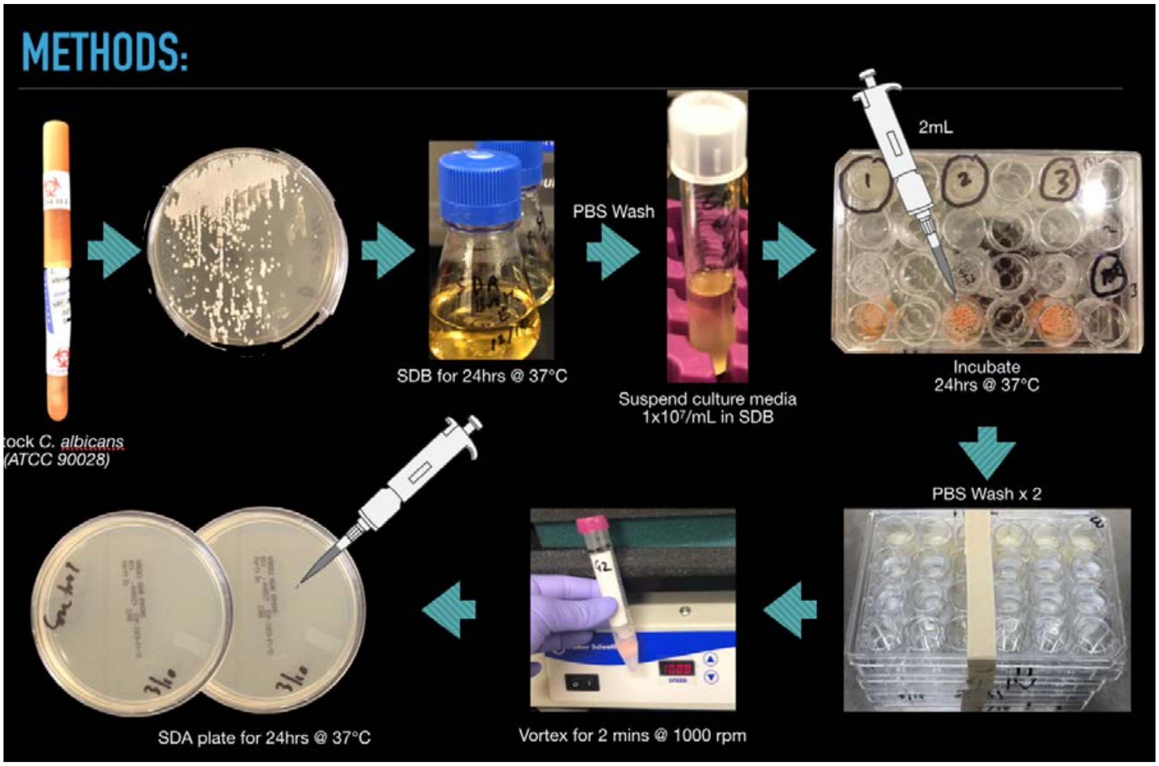
With continuous development and increasing interest in CAD/CAM manufacture, 3D additive technology will play a significant role in the future of dentistry, including complete and partial denture fabrication. Within the limitations of this *in-vitro* study, 3D-printed denture bases show great potential in digital dental clinical context, however, further investigations are needed to validate the findings from this study.

CHAPTER V: CONCLUSIONS

Current technology allows for complete, or partial, immersion into digital methods of denture fabrication. Additionally, new or modified resin materials with enhanced properties for CAD/CAM are being introduced. However, 3D-printed denture resins and their unique processing techniques are relatively new. Their mechanical and physical properties are not completely known. Although scientific publications from academia and manufacturers indicate that there is a potential for reducing microbial adherence to CAD/CAM manufactured dentures, the findings are not conclusive and in some instances contradictory. Within the limits of this study, there were no statistically significant differences in adhesion and formation of *C. albicans* among four denture base polymers tested. However, the in-vivo adherence mechanisms of microorganisms are complex, and *in-vitro* conditions as described were limited.

Further studies are recommended to include: larger sample size (to allow elimination the outliers), thermocycling (to simulate aging and understand longevity of each material), SEM imaging (to evaluate surface roughness), and validating adherence with a secondary method (e.g., XTT). This study and future studies are important, as 3D manufacturing has broad applications and will continue transforming dentistry.

APPENDIX: ADDITIONAL METHODS



- *C. albicans* (American Type Culture Collection 90028) was used, isolated and cultivated in Sabouraud dextrose broth for 24 hours at 37℃.
- The cells were harvested, washed twice with phosphate-buffered saline. The cell concentration was diluted to 10^7/mL in SDB.
- The specimens were placed in 24-well plates containing 2 ml of the adjusted culture and incubated at 37°C for 24 hours.
- Following incubation, the specimens was transferred to new plates and each specimen were washed in sterile PBS to remove non-adherent cells.
- The adherent cells were dislodged from the surface of the denture base by vortexing for 2 minutes at 1000rpm.
- The cell suspension was than plated on the agar (SDA) in duplicates.

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