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EFFECTS OF AGING ON ATTACHMENT OF *CANDIDA ALBICANS* TO

CONVENTIONAL HEAT-POLYMERIZED, CAD/CAM MILLED AND CAD/CAM

3D PRINTED ACRYLIC DENTURE RESIN BASES

by

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ABSTRACT

EFFECTS OF AGING ON ATTACHMENT OF *CANDIDA ALBICANS* TO CONVENTIONAL HEAT-POLYMERIZED, CAD/CAM MILLED AND CAD/CAM 3D PRINTED ACRYLIC DENTURE RESIN BASES

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Purpose: The aim of this study was to quantify the attachment of *Candida Albicans* (*C.albicans)* to conventionally fabricated (poly methyl methacrylate, PMMA), CAD/CAM milled and 3D printed denture resin bases and compare changes while the denture resin bases were subjected to thermal simulated aging processes. **Introduction:** The advent of Computer-Aided Design and Computer-Aided Manufacturing (CAD/CAM) milled and 3D printed resins has streamlined much of complex denture fabrication processes. The interaction between *C.albicans* an opportunistic fungal pathogen, and new CAD/CAM milled or 3D printed denture resin base surfaces are largely unknown. *C.albicans* is a major etiological factor of denture stomatitis in which symptoms include inflammation, swelling, and petechial hemorrhages. Stomatitis, if untreated, can lead to ill-fitting dentures, oral erythema, and dysphagia, ultimately diminishing the quality of life of patients. **Materials and Methods:** 184 standardized denture resin bases were divided into four material groups and sub-divided into three aging groups. Four material groups were conventional heat-polymerized PMMA (CHP), CAD/CAM milled by AvaDent (ADM), CAD/CAM 3D printed Dentca Denture Base II by Dentca (DDB), and Lucitone Digital Print 3D by Dentsply (LDP) and three aging groups were 0, 1 or 2 year aged simulations. *C.albicans* were grown and allowed to attach to resins for 24 hrs at 37℃. Adherent cells were quantified using XTT assay. Material groups and aging sub-groups were compared using four by three factorial analysis. All denture resin base samples were imaged by 150x stereoptical digital microscope before and after the thermal aging process. **Results:** Regardless of the fabrication technique or the material type of the denture resin base, statistical analysis suggests that no significant differences were seen in adhesion of *C. albicans* before or after thermal aging processes. However, several general trends were noted: (1) *C.albicans* attachment on DDB increased slightly with thermal aging, (2) *C.albicans* on LDP remained at the same level or showed a slight decrease during thermal aging. **Conclusions:** It was found that all four denture resin bases were equally stable up to 2 years of simulated thermal aging processes. Within the limitations of this study, we deduce that new denture materials and fabrication methods are as effective as the conventionally heat polymerized PMMA denture resin base in prevention of *C.albicans* attachment and have similar surface characteristics after thermal aging.

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1. DS: Denture Stomatitis
2. CAD/CAM: Computer-Aided Design and Computer-Aided Manufacturing
3. PMMA: Polymethyl-Methacrylate
4. *C. albicans: Candida albicans*
5. SLA: Stereolithography
6. DLS: Digital Light Synthesis
7. Clip: Continuous Liquid Interface Production:
8. PBS: Phosphate-buffered saline
9. SDB: Sabouraud dextrose broth
10. ATTC: American Type Culture Collection
11. ABS: Acrylonitrile Butadiene Styrene
12. XTT: sodium 3´-[1- (phenylaminocarbonyl)- 3,4- tetrazolium]-bis (4-methoxy6-nitro) benzene sulfonic acid hydrate

CHAPTER 1: INTRODUCTION

In 2019, there were 703 million people aged 65 years or over in the world. That number is projected to double to 1.5 billion in 2050.[1] As the world's population age, edentulism continues to be a challenging problem among oral healthcare providers. Complete denture fabrication is still considered a current, relevant, and cost-effective treatment option for edentulism. Douglass *et al.* estimated that 7.2 million more dentures will be made in 2020 compared to 1991. [2] A common side effect of denture wearing is denture-related oral soft tissue lesions which include denture stomatitis (DS). DS is an inflammatory process of the oral mucosal areas that underlie a removable denture and may affect a wide range of denture wearers from 15% to more than 70% from various studies. [3] Shulman *et al.* found that 28% of denture wearers develop DS [4]. *Candida albicans* (*C. albicans)* were shown to be the most prevalent isolated species in DS. [3,5,6] *C. albicans* has the ability to grow on specific surfaces such as denture acrylic [7] by forming biofilm which enhances its adhesion and prevents the cells from dislodging from the surface. [8] A study by Ramage *et al.* described how surface roughness or defects of the denture acrylic could promote attachment and networks of *C. albicans* embedding into the acrylic surface. [9] Bidra *et al,* found that decreasing the surface porosity of the denture resin base decreases the adherence of *C. albicans.* [10]

Dentures have been fabricated conventionally via heat-cured polymerization of poly methyl methacrylate (PMMA) since 1937. [11-15] Generally, the heat-cured PMMA contains a large number of porosities due to shrinkage during the polymerization process. [14] Recently a new method of denture fabrication has been developed using subtractive manufacturing through Computer-Aided Design and Computer-Aided Manufacturing (CAD/CAM) technology. Denture bases are now milled from pre-polymerized pucks of resin (AvaDent, Global Dental Science

LLC, Scottsdale, AZ). [10,16,17] Shrinkage of the final prosthesis is limited because the puck is under high pressure and heat during its manufacturing process. This new process allows for a decline in residual free monomer which results in a decrease in the porosity of the denture base surface. [10,16,17] A recent study showed that *C. albicans* adhered to the CAD/CAM denture resin base with a lower affinity than to the conventional heat-cured PMMA denture base. [18]

In addition to the milled method, there is another CAD/CAM fabrication method which employs 3D printing or additive manufacturing (rapid prototyping) processes. The denture printing methods include using stereolithography (SLA) and the most novel method which is by digital light synthesis (DLS) with continuous liquid interface production (CLIP). SLA is a type of liquid resin layered manufacturing, in which a scanned dental structure image and a virtual design of the final prosthesis are combined into one. An ultra violet laser polymerizes the surface of liquid photo-polymerizing resin on contact as the laser moves in cross sectional increments of 1mm thus producing a 3D dental prosthesis. [17,19-25] DLS propelled by CLIP (Carbon3D, Redwood City, CA), uses an oxygen-permeable window which inhibits layered polymerization allowing for continuous, layerless, isotropic printing. UV light is projected into the resin reservoir via the oxygen-permeable window. A support plate rises when the denture resin solidifies as a series of UV images are projected. [26-29]

Proprietary types of denture resins namely: Dentca 3D Denture Base (Dentca, Inc.) and Lucitone Digital Print 3D Denture Resin (Dentsply Intl.) can be used in conjunction with 3D printers to fabricate denture resin bases[30] The Dentca 3D denture resin base is composed of urethane methacrylate-based photopolymerized resin. [31] In the recent past, the Dentca denture resin base was mainly utilized for fabrication of interim prosthesis or as a trial denture, and rarely as a definitive final denture. [25] However, with recent FDA approval, it is classified as a

Class II long-term biocompatible denture resin base. The Dentca denture resin base can be printed with the Formlabs Form 2 desktop stereolithography 3D printer. The second 3D printer specific denture resin base, the Lucitone Digital Print 3D, is composed of urethane methacrylate photosensitive polymer liquid resin and it can be used to print a high impact denture. [32] This liquid resin is polymerized by the ultraviolet light employing CLIP technology utilizing the Carbon M2-Series printer workflow.[26] The quality, service-life, surface characteristics, and biofilm formation and attachment of 3D printed dentures are largely unknown.

Even with a relatively short track record, CAD/CAM milling and CAD/ CAM 3D printing technology of manufacturing denture resin bases demonstrate several promising advantages compared to the conventional compression molding and heat processing method. [10,30]. These advantages include enhanced physical properties such as more hydrophilicity aka wettability of the denture surface [33] which aid in enhanced retention especially for patients with salivary dysfunction. [33-36] CAD/CAM denture resin bases also have a smoother intaglio surface than conventional PMMA. [33,37,38] Previous studies have shown that milled CAD/CAM denture resin bases have different surface characteristics than conventional denture resin bases. [38] The milling process and milling burs leave ripples on the cameo and intaglio of milled surfaces, and the degree of roughness depends on the types and quality of the milling tools. [37,38] Clinically, the cameo surface is polished with various compounds for the patient's comfort, however, the intaglio surface is not polished to retain desirable retention. [37] Several previous studies have reported that CAD/CAM milled denture resin bases are smoother than conventional denture resin bases. [38-40] Generally, oral microbial adhesion decreases on smooth surfaces compared to rough surfaces, including denture resin bases.[18,41] *C. albicans* is a hydrophobic organism therefore it can adhere more readily to denture surfaces that have hydrophobic properties. [42,43] Interestingly, in a study by Alp *et al*, when three prepolymerized CAD/CAM resins were compared to conventional heat-cured PMMA after liquid bath in coffee and thermal aging simulation, there was no significant difference of surface roughness among those four resins which suggests minimal porosity and high polishability of CAD/CAM denture resin bases were not critical. [41] Changes in oral microbial adhesion, if any, on CAD/CAM denture resin base surfaces after thermal aging simulation are not yet known.

Another major advantage of CAD/CAM digital technology is it is extremely easy to duplicate or redesign an existing denture using previously stored digital files. Furthermore, these files can be easily transmissible to a lab anywhere in the world to be milled or 3D printed. Dentures are replaced, duplicated or redesigned for various reasons. The service life also varies. The American College of Prosthodontists, recommends replacement after 5 or more years of service. [44] According to Lewis *et al*, maxillary and mandibular complete dentures should be replaced every 6.5 years. [45] Delta dental insurance co. states that replacement of a complete denture is covered every 7 years [46], however the Alberta recently extended health benefits plan covers denture replacement every 5 years. In a study by Hoad-Reddick *et al*. concluded that on average after 5 years of denture wear, 40 percent of dentures needed replacement, and 80 percent will need replacement after 10 years. [47] If dentures are fractured, not maintained properly, or misplaced, the frequency of denture replacement may be shorter than the average service life which reinforces the strength of the CAD/CAM digital technology.

With many potential advantages of CAD/CAM milled and 3D printed dentures, there is a gap in the current knowledge as to how would *C. albicans* respond to different denture materials and fabrication methods as the materials are normally aged in the oral cavity. The purpose of this study was to quantify the attachment of *C. albicans* to conventionally heat-polymerized

(PMMA), CAD/CAM milled and 3D printed denture resin bases and compare changes after the resin bases were subjected to thermal simulated aging processes of 0, 1 or 2 years. The null hypothesis was there would be no difference in *C. albicans* attachment based on material types or after thermal simulated aging processes.

CHAPTER 2: MATERIALS AND METHODS

Denture resin base sample preparation:

The study samples were designed using the 2D CAD Drafting and 3D design by SOLIDWORKS computer software. The virtual design of the discs with the dimensions 87mm (l) x 10mm (w) x 2mm (d) and 1mm cut lines incorporated into the final design (Fig. 1). These 1mm cut lines allowed for precision in obtaining uniform sample dimensions of the samples with minimal effort (either snap off or cut off). The file of the design was formatted to the Standard Tessellation Language (STL) for the CAD/CAM manufacturing. The materials used were 4 commercially available denture resin products. The conventional heat-polymerized lucitone 199 PMMA (CHP), AvaDent CAD/CAM Denture Base Puck (ADM), and then 2 different materials and printers for the 3D printed; CAD/CAM 3D printed Dentca Denture Base II by Dentca (DDB), using the Formlabs Form 2 desktop SLA 3D printer and Lucitone Digital Print 3D by Dentsply (LDP) using the Carbon M2 DLS, 3D printer and were prepared according to the manufacturer's instructions.

For this study a total of 184 samples were fabricated. Within the 4 materials the samples were subdivided into 3 different aging groups (0) as the control, 1Y representing 1 year of aging (10,000 thermocyles) and 2Y representing 2 years of aging (20,000 thermocyles). 40 samples were included in the control group then the additional 144 samples, were randomly and equally divided into the 2 thermocycle groups (10,000 and 20,000 cycles) per material; with 36 samples per material group and 18 samples per thermocycle group.

This sample size was used in order to be able to conduct a 4×3 factorial analysis.

The CHP denture resin base samples (Lucitone 199; Dentsply Int.) were processed using the conventional compression mold technique. The designed disc sample was milled in wax (ProArt Wax Disc for Zenotec; Wieland Dental+Technik GmbH & Co. KG) and was used in the conventional investment process, cured in long polymerization cycle; 9 hours in a water bath at 73°C ±1°C, followed by 30 minutes in boiling water at 100°C as recommended by the manufacture. After the deflasking procedure, excess flash was removed with carbide E-cutter Kit (1108.11 BrasselerUSA) and light polished was applied with silicone resin base acrylic polishing bur (BrasselerUSA). The CHP samples were washed and stored in distilled water (to minimize the influence of residual monomers or toxic constituents on cell viability), and sterilized under the ultraviolet light unit for 10 minutes. The thermally aged group samples were aged according to group, washed and stored in distilled water, and sterilized under the ultraviolet light.

To prepare the ADM, denture resin base samples, the STL file of the virtual design was nested and calculated for the tool path in the Zenotec software (Wieland Dental+Technik GmbH & Co. KG). AvaDent CAD/CAM Denture Base Puck was inserted and the samples were milled according to the design. Following the milling process, the samples were sectioned and the excess/rough edges were polished with silicone resin base acrylic polishing bur (BrasselerUSA). The control resin base samples were washed and stored in distilled water, and sterilized under the ultraviolet light. The thermally aged group samples were aged according to group and then were washed and stored in distilled water, and sterilized under the ultraviolet light.

To prepare CAD/CAM 3D printed denture resin bases, the STL file were sent to two different facilities with instructions to print designed samples. The DDB samples were prepared at the Walter Reed National Military Medical Center 3D Medical Application Center. The DDB samples were printed following the Formlabs Form 2 desktop stereolithography 3D printer protocol, and the samples were returned fully cured/processed without sectioning or polishing. The LDP samples were prepared at Absolute Dental Services in Durham, NC. The LDP samples were printed following the Carbon M2-Series printer workflow and again, the samples were fully cured/processed without sectioning or polishing. Next, the samples were verified in their dimensions, sectioned, and the excess/rough edges were polished with silicone resin base acrylic polishing bur (BrasselerUSA). The control denture resin base samples were washed and stored in distilled water, and sterilized under the ultraviolet light. The thermally aged group samples were aged according to group and then were washed and stored in distilled water, and sterilized under the ultraviolet light.

Note: In this study, aggressive high polish procedures were not intentionally accomplished in order to mimic the tissue-baring intaglio surface of the denture resin base.

Aging Preparation:

Samples were randomly divided into 2 groups. Samples for each group were inserted into a holding cassette (Fig 2) which was designed using 2D CAD Drafting and 3D design by SOLIDWORKS computer software and printed using acrylonitrile butadiene styrene (ABS) on the Statasys Fortus 250MC. Thermocycling commenced in the cassette for 10,000 or 20,000 cycles respectively using the (SD mechatronik GMBH) thermal cycler with temperatures from 5 ± 2℃ to 55 ± 1 °C with a 60 second dwell time [48,49].

Candida albicans preparation:

C. albicans (American Type Culture Collection: 90028) was the strain utilized, isolated and cultivated in Sabouraud dextrose broth (SDB) for 24 hours at 37°C. The cells were harvested, washed twice with phosphate-buffered saline (PBS). The cell concentration was adjusted to $1x10^7$ mL in SDB.

The samples were placed in 24-well plates (1 denture resin base disk/well) containing 2 ml of the inoculum and incubated at 37°C for 24 hours. Following incubation, the samples were transferred to new 24-well plates and each sample was washed in sterile PBS to remove non-adherent cells. After the samples were washed with PBS they were ready for quantification of the adherent cells using the cell proliferation assay; XTT assay.

Optical density- XTT Assay:

XTT solution (1mg/mL in PBS) was prepared, filter sterilized through a 0.22 µm pore size filter, and stored at -70°C. Menadione solution (0.4mM in acetone) was prepared immediately before the assay. Prior to each assay, XTT solution was thawed and mixed with menadione solution at a ratio of 5 to 1 by volume (XTT/menadione reagent).

A 1.5 ml XTT/menadione reagent was added to the wells and incubated in the dark for 2 hours at 37°C. Following incubation, 100 µL of the solution was transferred to new wells in a 96-well microtiter plate and the color change in the solution was quantified using the Eliza scanning multi-well spectrophotometer reader at 492nm using a 96 microtiter plate reader.

CHAPTER 3: RESULTS

To test the hypothesis that thermal aging leads to increased fungal attachment to denture surfaces and the degree of the damage is fabrication method dependent, thermocycling was used to simulate the intraoral denture aging process. According to Lira *et al*, 20,000 thermal cycles in alternating water baths at 5 ± 2°C and 55 ± 1°C with a 60-second dwell time represents a realistic simulation of an individual's daily thermal changes during approximately 2 years [49]. "It replicates a person's exposure to oral temperature changes 10 times a day for their eating (meals, water, coffee, ice cream etc.) with three thermal cycles per each procedure during approximately 2 years" [48,50]. In order to simulate the adequate number of thermal changes a person would encounter in a day the thermocycling times chosen for this study are for 1 year or 10,000 thermal cycles and 2 years or 20,000 thermal cycles. Four commercially available denture resin base materials were evaluated in this in vitro study (Table 1) and all samples were prepared according to the manufacturer's instructions.

Quantification of C. albicans before and after simulated thermal aging process

After 1 or 2 years of simulated aging, we did not observe statistical differences in candida attachment among the four denture resin base materials. Focusing on the control group, there were slight differences among the four denture resin base materials, especially the two printed materials. However, these differences were not statistically significant (Fig 3).

Comparison of C. albicans attachment among material types

A 4x3 factorial statistical analysis was performed and there was no significant interaction between the denture resin base materials and the aging cycles (p-value =0.91>.05). However, several general trends have been observed. *C. albicans* attachment on CHP and DDB printed increased slightly with thermal aging. In addition, *C. albicans* attachment on ADM and LDP remained at the same level or showed a slight decrease during thermal aging. Lastly DDB, had the least *C. albicans* attached and LDP had the most, at the starting point, without thermal aging; however, this difference became negligible after 2 years of simulated thermal aging (Fig 4).

Surface characterization of denture resin base samples before and after simulated thermal aging

Fig 5 represents the samples under a high powered stereoptical digital light microscope. In support of our *C. albicans* attachment results, we observed no discernable surface changes or damages after the samples were thermally aged.

In summary there were no statistically significant differences among the four types of denture resin bases at thermal aging 0, 1, or 2 years. *C. albicans* attached to all four types of denture resin bases similarly. The surfaces of the four denture resin bases were intact even after 1 or 2 years of simulated thermal aging processes. Although not statistically significant, several general trends were observed. *C. albicans* attachment on CHP and DDB increased slightly with thermal aging. *C. albicans* attachment on ADM and LDP remained at the same level or showed a slight decrease during thermal aging. Although not statistically significant, at the starting point (no thermal aging), DDB had the least candida attached and LDP had the most. This difference became negligible after 2 years of thermal aging.

CHAPTER 4: DISCUSSION

Regardless of the fabrication technique or the material type of denture resin base, statistical analysis, suggests that no significant differences were seen in adhesion and formation of *C. albicans.* The null hypothesis which was that there will be no difference in *C. albicans* attachment based on material type or after thermal simulated aging was accepted. As seen in Fig 4, it is concluded that the new materials; ADM, DDB, LDP were comparable to the CHP denture resin base in terms of *C. albicans* attachment of the thermally aged samples up to 2 years or 20,000 thermal cycles of simulated usage.

In previous studies comparing conventional to CAD/CAM milled Al-Fouzan *et al* [18] found that the more hydrophilic nature of CAD/CAM milled dentures may make them less susceptible to microbial adhesion than the conventional heat-activated resins. Steinmassel *et al* [37] found that when immersed in saliva, CAD/CAM milled and injection molded dentures showed an overall improved fit in the palatal and post-dam areas when compared to conventionally fabricated complete dentures. They attributed these results to the undulating ripples created in the intaglio surface by the milling machine that can provide adhesive retention when filled with saliva.

In a study by Murat *et al*.[40], they compared the amount of adherent *C. albicans* to different CAD/CAM PMMA based polymers and conventional heat-polymerized PMMA after thermal cycling. First, the authors evaluated the surface roughness under the scanning electron microscopy (SEM) and reported that there are significant differences between the conventional and CAD/CAM PMMA based polymers. No difference was found between different CAD/CAM PMMA based polymers (three different brands), however conventional PMMA polymers exhibited a more porous surface and surface irregularities. The authors also observed that long-term thermal cycling may also affect the surface roughness, which could alter the surface energy/hydrophobicity of the resins, however, no clear relationship was seen between 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 be preferable to reduce *Candida*-associated denture stomatitis in long-term use.

In a study comparing CAD/CAM milled to 3D printed, Meirowitz *et al* [51] showed that manufactured denture base acrylic resin discs using 3D printing significantly increased *C. albicans*' adhesion as compared with the conventional method of heat curing, whereas computerized milling significantly decreased the microbial adhesion. Our results partially support this study, in that our thermal aging control group did demonstrate that LDP initially had the most *C. albicans* adhesion but DDB had the least and then after thermal aging the difference became negligible.

In a study by Shim *et al* [42] it was shown that the adhesion of microbes to 3D printed denture bases is dependent upon the printing angle. If the denture is printed at a 90-degree angle there is less adhesion of *C. albicans* than if it was printed at 0 or 45 degrees. The issue with the denture being printed at 90 degrees however is that the flexural strength of the denture decreases.[42] Advantages of 3D printing are that it is more cost effective and economical. Another advantage, highlighted in a recent in vivo study by Kim *et al*, showed that 3D printed dentures had less post insertion sore spots than the conventionally fabricated. [52]

Based on data collected from the corporate dental system, CDS, which is the web-based application that serves as the Department of Defense's current electronic dental record system, the data shows that dentures are being made for our service members across the branches. Fig 6 represents the number of dentures fabricated for active duty service members from 2015 to 2020. There were 738 dentures fabricated for Active Duty Army personnel, 124 for Navy Personnel, 41 for Air force personnel and 21 for the Marine Corps. Complete denture fabrication is still considered a current, relevant, and cost-effective treatment option for edentulism found in the general population and our service members.

Limitations of the present study include that only one species of *C. albicans* was used. There are a multitude of intraoral bacteria and it is uncertain how these other species may act to assist the *C. albicans* in attaching to various surfaces. Next, we used thermocycling to simulate aging. However, dentures in the oral cavity are exposed to various other conditions, including chemical and mechanical aging processes which were not implemented in this study. Lastly, we limited our simulated aging process to 2 years; however this was based on guidelines for the care and maintenance of complete dentures published by the American College of Prosthodontists, which states that “dentures should be checked annually.”[53]

CHAPTER 5: CONCLUSIONS

In conclusion, it has been shown that CAD/CAM milled and 3D printed denture resin bases have the advantages of time saving, they are easy to reproduce and have a better accuracy and fit than the conventional denture resin bases. [25,54] This study showed that AMD, DDB and LDP denture resin bases have comparable thermal aging effects when compared to the CHP PMMA as well as similar attachment levels of *C. albicans* signaling a clinical implication that these newer materials are comparable to the "gold standard" of the conventional PMMA denture resin base.

Future research should address using different strains of *C. albicans,* incorporating saliva, increasing the thermocycle times, or including other simulated aging such as chewing or brushing. The next level of research is an in vivo study of these different denture resin base materials and varying fabrication methods.

Within the limitations of this study we can conclude:

1. CAD/CAM milled and CAD/CAM 3D printed materials exhibit comparable aging affects as the Conventional PMMA material.
2. CAD/CAM Milled and CAD/CAM 3D Printed materials demonstrate similar levels of *C. albicans* adhesion to the Conventional PMMA material.

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Table 1. Denture base polymers tested

Material	Code	Polymer Type	Method of Fabrication	Manufacture
Lucitone 199 (Conventional heat-polymerized)	CHP	PMMA- High impact	Heat and Compression	Dentsply Intl.
AvaDent CAD/CAM Denture Base Puck	ADM	PMMA-Pre-polymerized	CAD/CAM Five-axis milling	Global Dental Science
DENTCA Denture Base II	DDB	Methacrylate-based photopolymer liquid resin	CAD/CAM 3D-printed	Dentca, Inc.
Lucitone Digital Print 3D Denture Resin	LDP	Urethane methacrylate photosensitive polymer liquid resin	CAD/CAM 3D-printed	Dentsply Intl.

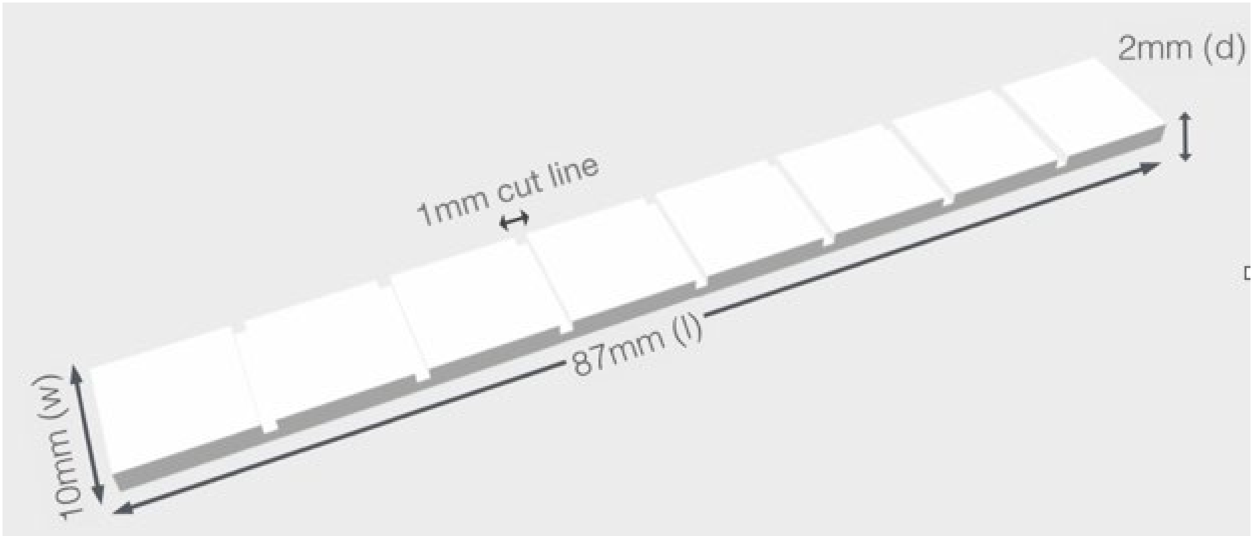

Figure 1. Denture resin base sample design. Standardized sample dimensions 87mm (l) x 10mm (w) x 2mm (d)

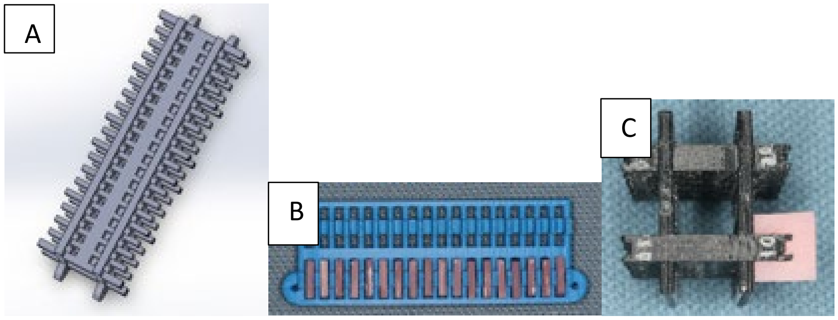

Figure 2. Custom-made sample holder. Virtually designed sample cassette holder (A). Printed sample cassette holder with 18 samples in place (B). Top view of sample in place (C).

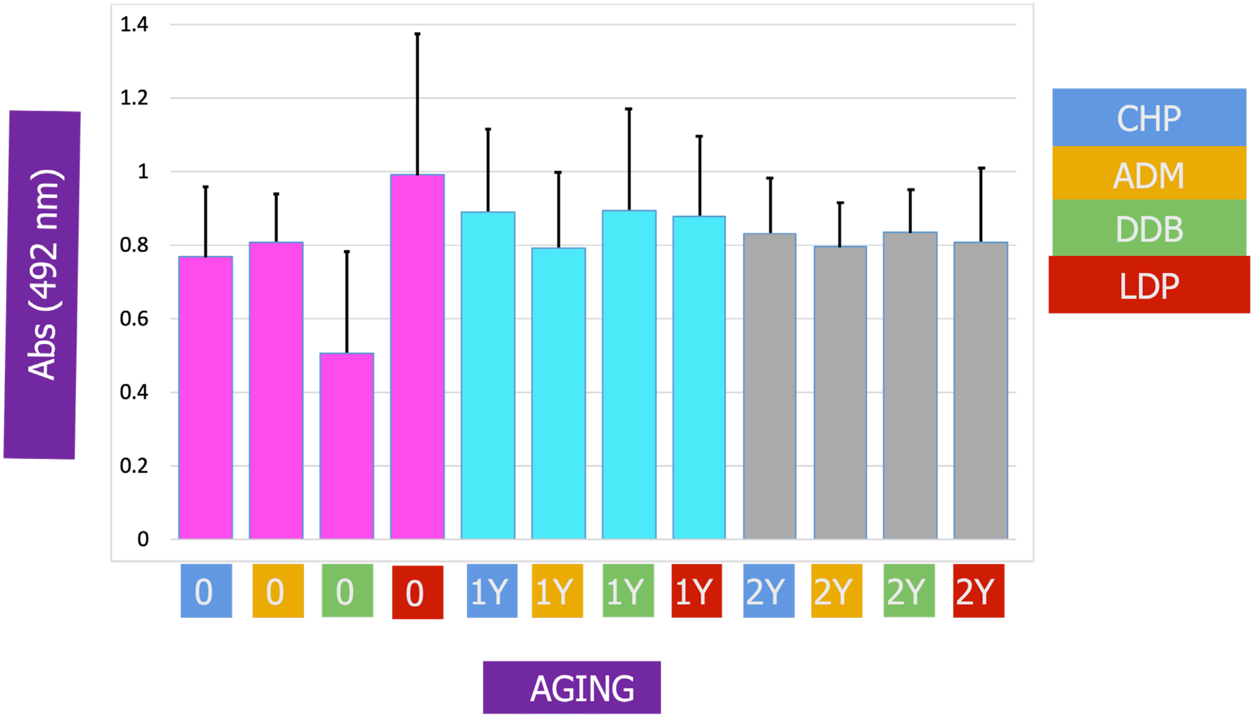

Figure 3. Boxplot of *C.albicans* attachment by denture aging. This is a graph representing quantification of *C.albicans* attachment. On the y axis is the absorbance at 492 nm representing the optical density generated from the spectrophotometer. On the x axis are the aging cycles at the control or (0), 1 year and 2 years representing thermal aging in the mouth. The purple bars graphs represent aging cycle (0), the light blue bars graphs represent aging cycle 1 year and the grey bars graphs represent aging cycle 2 years. The error bars represent the standard deviations. After 1 or 2 years of simulated aging, no statistical differences in *C.albicans* attachment among the four denture materials were observed. In the control group, there were slight differences among the four denture materials, especially the two printed materials. However these differences were not statistically significant.

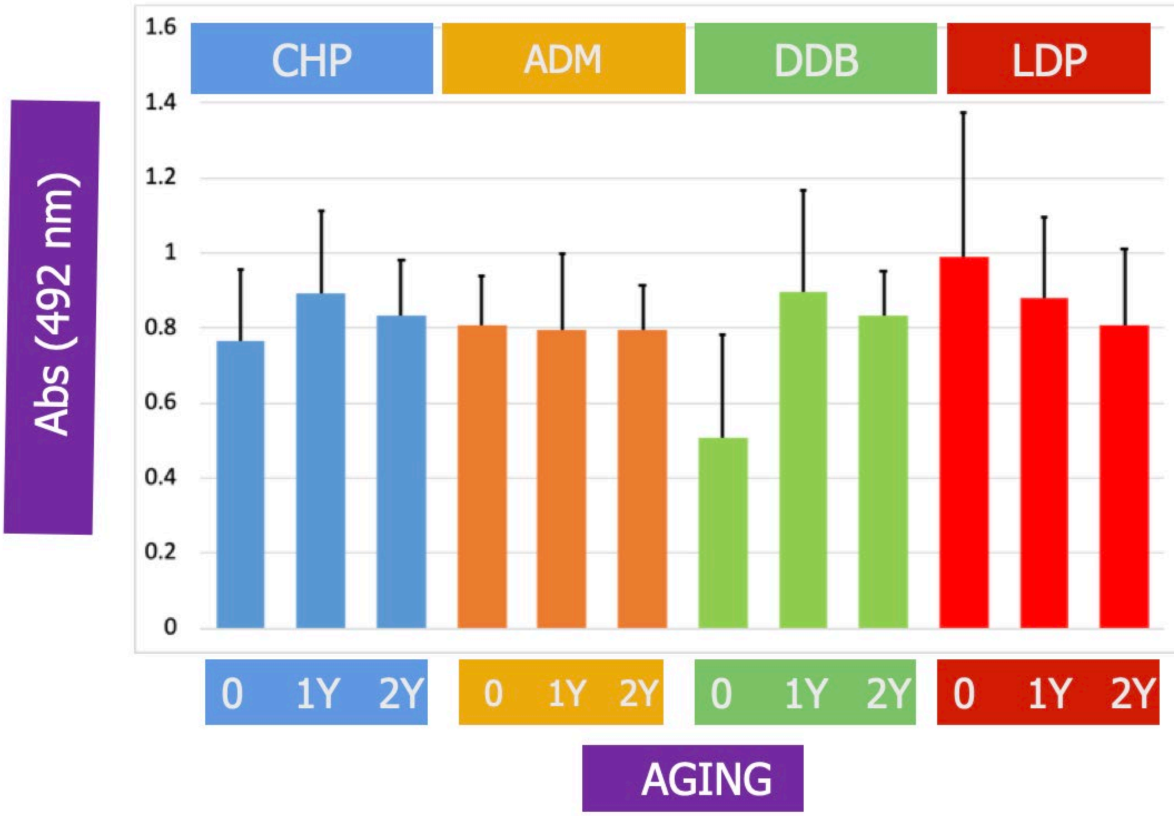

Figure 4 Boxplot of *C.albicans* quantification by denture material. This is a graph when quantification of *C.albicans* attachment is grouped by material type. The blue bars represent the conventional heat-polymerized (CHP), the orange bars represent AvaDent CAD/CAM milled Denture Base Puck (ADM), the green bars respresent CAD/CAM 3D printed Dentca Denture Base II by Dentca (DDB), and the red bars represent CAD/CAM 3D printed Lucitone Digital Print TM 3D by Dentsply (LDP). On the y axis is the absorbance and on the x axis are the aging cycles at the control or (0), 1 year and 2 years representing thermal aging. The error bars represent the standard deviations. No significant interaction between the denture materials and the aging cycles were observed. *C. albicans* attachment on CHP and DDB increased slightly with thermal aging. *C. albicans* attachment on ADM and LDP remained at the same level or showed a slight decrease during thermal aging. Lastly, DDB had the least *C. albicans* attached and LDP had the most, at the starting point, without thermal aging; However, this difference became negligible after 2 year simulated thermal aging.

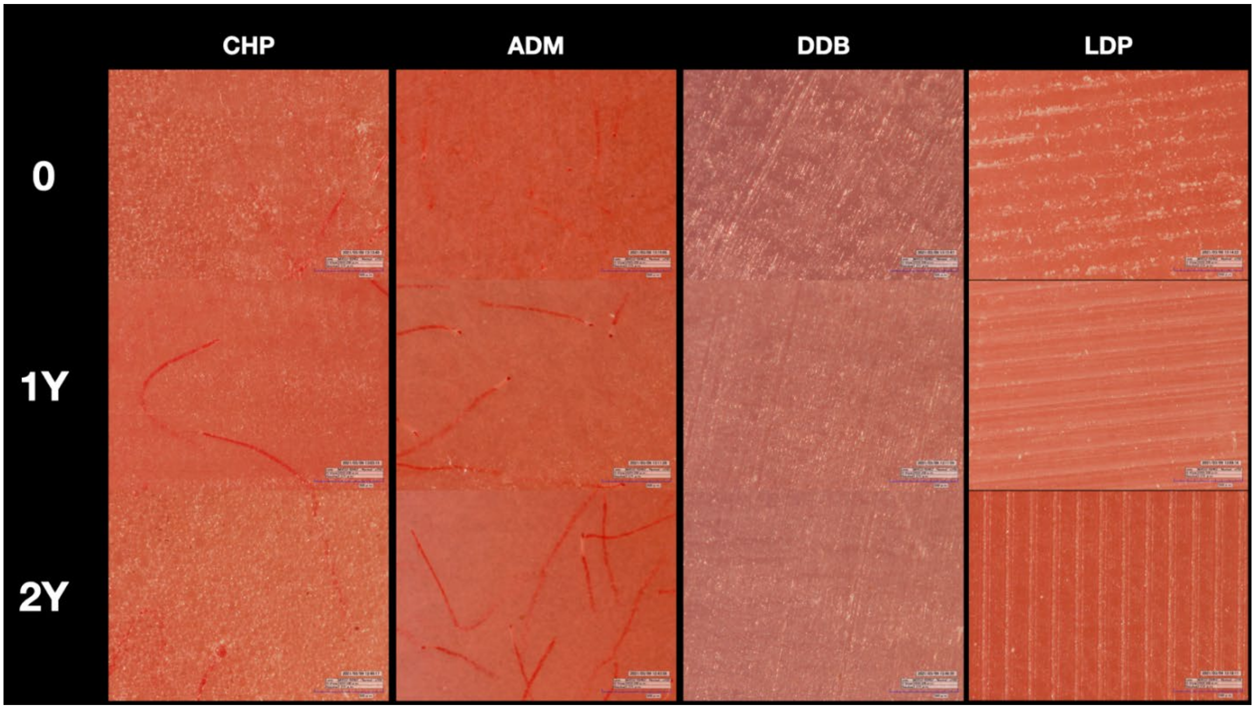

Figure 5. High powered stereoptical digital light microscope images of denture surfaces. On the y axis are the aging cycles at the control or (0), 1 year and 2 years representing thermal aging. On the x axis are the material types (CHP,ADM,DDB and LDP) No discernable surface changes or damages after the samples were thermal aged. (Hirox parameters: X150, H-View: 2033 µm, Resolve:1.2 µm, and bar =500 µm)

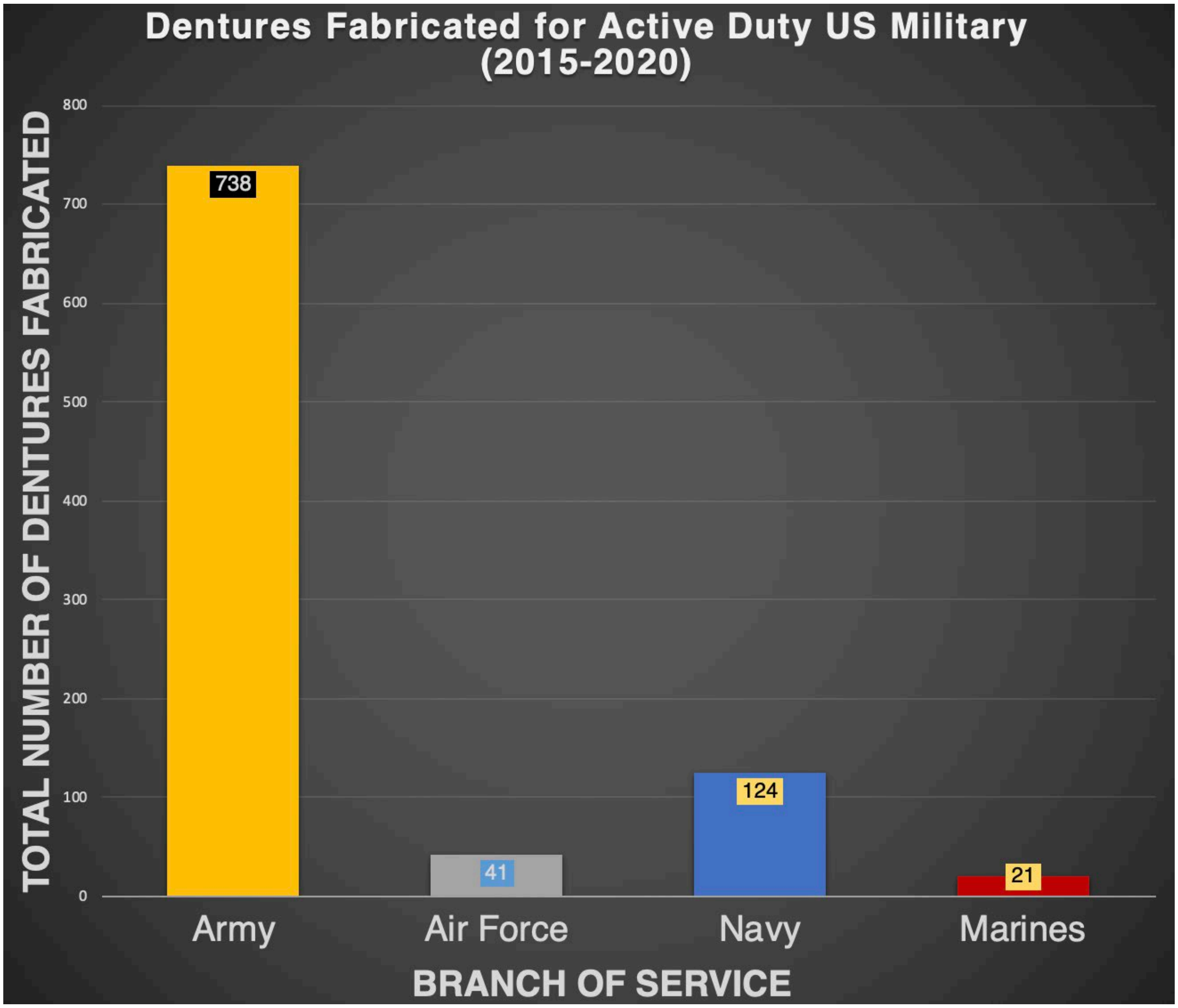

Figure 6. Dentures fabricated for Active Duty US Military. This graph represents the number of dentures fabricated for active duty service members from 2015 to 2020. On the y axis is the total number of complete maxillary and mandibular dentures fabricated and on the x axis is the branch of service.