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Candida Albicans Adhesion of New-generation Denture Base Materials

Candida Albicans'ın Yeni Nesil Protez Kaide Materyallerine Adezyonu

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ÖZET

Amaç: Yaklaşık %65 protez kullanıcısını etkileyen protez stomatiti, yaygın bir oral kandidiyazis belirtisidir. Dijital diş hekimliğinin gelişmesiyle beraber protez kaide materyali olarak daha güncel materyaller kullanılmaktadır. Candida albicans'ın adezyonunun yeni nesil protez kaidelerine etkisi incelenmemiştir. Bu araştırma, çeşitli üretim yöntemleriyle oluşturulan farklı dental polimerlere C.albicans'ın tutulumunu ve termal siklusun etkisini incelemeyi amaçlamaktadır.

Gereçler ve Yöntem: Mikrobiyolojik testler için toplam 60 disk örneği (10×2 mm) üretildi. Örnekler, her malzeme grubu için iki alt gruba ayrıldı (n = 10). Üç farklı protez kaide malzemesi farklı teknikler kullanılarak üretildi: 3 boyutlu (3D) baskıyla üretilen protez kaide resini, Formlabs (FL); geleneksel ısı ile polimerize edilmiş akrilik resin, Meliodent (MD); ve bilgisayar destekli tasarım/bilgisayar destekli üretim (CAD/CAM) teknolojisi kullanılarak üretilmiş pre-polimerize edilmiş polimetil metakrilat (PMMA) diski, Ivobase (IB). Termal döngü öncesi ve sonrası numuneler test edildi (5000 döngü, 5 °C/55 °C). Örneklerin C.albicans tutulumu mikroskopta incelendi. Tüm grupların yüzey görüntüleri taramalı elektron mikroskobu (SEM) kullanılarak değerlendirildi. Verileri incelemek için post-hoc Tukey testi ve iki yönlü varyans analizi kullanıldı.

Bulgular: CAD/CAM ile frezelenmiş grup ve 3D baskı grubu, ısı ile polimerize edilmiş akrilik resine kıyasla önemli ölçüde daha az C.albicans tutulumu gösterdi. Termal döngünün mikrobiyal tutuluma etkisi, test edilen tüm gruplar için önemsiz bulundu.

Sonuç: Kandida enfeksiyonları ve buna bağlı protez stomatiti, geleneksel ısı ile polimerize edilmiş akriliğe kıyasla yeni nesil protez kaide materyallerinde daha az görülmektedir. Mikrobiyal tutulumu azaltmak için, 3D baskıyla üretilen ve CAD/CAM ile frezelenmiş protez kaide materyalleri daha iyi bir tercih olabilir.

Anahtar Kelimeler: 3D baskı, Candida albicans, protez kaidesi, termal siklus

ABSTRACT

Aim: Denture stomatitis, affecting approximately 65% of denture wearers, is a common symptom of oral candidiasis. With the advancement of digital dentistry, more contemporary materials are being used as denture base materials. The effect of Candida albicans adhesion on new-generation denture bases has not been investigated. This study aims to examine the adhesion of C. albicans to various dental polymers produced by different manufacturing methods and the effect of thermal cycling.

Materials and Methods: A total of 60 disk samples (10×2 mm) were produced for microbiological tests. The samples were divided into two subgroups for each material group (n = 10). Three different denture base materials were produced using different techniques: 3 dimensional (3D) printed denture base resin, Formlabs (FL); conventional heat-polymerized acrylic, Meliodent (MD); and milled pre-polymerized polymethyl methacrylate (PMMA) resin disc manufactured using computer aided design/computer aided manufacturing (CAD/CAM) technology, Ivobase (IB). Before and after thermocycling, specimens were tested (5000 cycles, 5 °C/55 °C). The adhesion of C. albicans on the samples was examined under a microscope. Surface images of all groups were evaluated using scanning electron microscopy (SEM). Post-hoc Tukey test and two-way analysis of variance were used to analyze the data.

Results: The CAD/CAM milled group and the 3D printed group showed significantly less C.albicans adhesion compared to the heat-polymerized acrylic resin. The effect of thermal cycling on microbial adhesion was found to be insignificant for all groups tested.

Conclusion: Candida infections and associated denture stomatitis are less common in new-generation denture base materials compared to conventional heat-polymerized acrylic. To reduce microbial adhesion, denture base materials produced by 3D printing and milled by CAD/CAM could be a better choice.

Keywords: 3D printing; Candida albicans; denture base; thermal cycling

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INTRODUCTION

The porous structure of denture bases sometimes makes them vulnerable to the buildup of microbial biofilms. The adherence of bacteria to the denture surface is the initial step in the creation of biofilms. Opportunistic pathogens like Candida albicans, which are linked to precancerous lesions and oral infections (1), can be found in this microbial biofilm (2). Candida-infected leukoplakia is associated with a greater risk of cancer (3). Denture prosthesis users have a prevalence of denture stomatitis that ranges from 15% to over 70%. Denture stomatitis is caused by pathogenic Candida infection, poor denture cleanliness, and persistent denture use. C.albicans has the ability to stick to the denture's acrylic foundation, which can cause excruciating oral mucosal inflammation linked to contaminated dentures (4,5).

The most commonly used prosthetic base material is polymethyl methacrylate (PMMA) (6). PMMA, polymerized with heat, is preferred for its cost-effectiveness, easy availability, biocompatibility, aesthetic properties, lightness, ease of manipulation, and repairability. However, a number of variables, including the powder-to-liquid ratio, the polymerisation technique (fast or slow curing), the storage conditions of the material and the skill of the technician, affect how long acrylic resin-based prosthetic bases last (6-10). Nevertheless, the search for an ideal prosthetic base material continues due to weak physical properties such as poor compatibility with the tissue due to thermal shrinkage of heat-polymerized acrylic prosthetics, (11) allergic reactions (9) caused by residual monomers, wear resistance, insufficient surface hardness, and low durability (6,7).

With the development of digital techniques, complete prostheses can now be created from fully or partially polymerized acrylic disks by additive (using three-dimensional printers to manufacture acrylic resins) or subtractive (milling) techniques. In comparison with the traditional methods, CAD/CAM technology enables the manufacturing of dental prosthetics with less discomfort to the patient, in a shorter time, and with high precision-fit. It also allows for the direct replication of existing prosthetics (12,13).

In the production of next-generation prosthetic bases, milling techniques are more common than 3 dimensional (3D) printed (12). The accuracy of milled prosthetics depends on the milling tools (size and number of milling tips) and the materials used (14,15). Structural defects are reduced in materials obtained through milling (14,16). Prosthetics produced by subtractive methods require large amounts of raw material and generate significant waste (17).

Manufacturing through 3D printing involves building up material layer by layer. Compared to subtractive systems, it's often more cost-effective because there's less material waste and no wear on tools (18). In addition, 3D printing makes it possible to create multiple objects at the same time and to produce complex, large designs (19,20). While 3D-printed full prosthetics offer a promising option for treating complete tooth loss, their widespread clinical adoption has not yet occurred.

The oral environment is dynamic due to temperature changes, making it crucial to mimic these conditions when testing material properties. Therefore, thermal cycling is the most preferred test method to simulate the oral environment.

When we review the literature, there are very few studies that assess the microbiological properties of newly developed 3D-printed and milled denture base materials. However, there is no study investigating the effect of aging through thermal cycling on Candida adhesion in the next-generation bases. Therefore, the purpose of this study is to examine the adhesion of Candida, the most commonly encountered fungal infection in denture patients, to new-generation denture base materials and evaluate how the process of aging through thermal cycling affects Candida adhesion. Our first hypothesis is that there will be differences in Candida adhesion among denture base materials. Our second hypothesis is that Candida adhesion will increase in all samples after the thermal cycling process.

MATERIALS AND METHODS

A total of 60 disc specimens were fabricated for microbial testing, each measuring 2 mm in thickness and 10 mm in diameter (21). The specimens in each group, further divided into two subgroups: one subjected to thermal cycling and the other not (n = 10/group). The specimens were manufactured with 3 different production techniques. IvoBase CAD (IB) (Ivoclar Vivadent, Schaan, Liechtenstein) produced from prepolymerized PMMA disc by CAD/CAM milling technology; formlabs denturebase (FL) (Somerville, MA, USA) produced from denture base resin by 3D printing technology and Meliodent (MD) (Kulzer, Berkshire, Germany) heat-polymerized acrylic resin produced by conventional method are the materials used in the study (Figure 1).

For the CAD-CAM specimens, the Fusion 360 CAD software program (Autodesk, headquartered in Mill Valley, CA, USA) was utilized to design a 3D model of a disk measuring 10 mm × 2

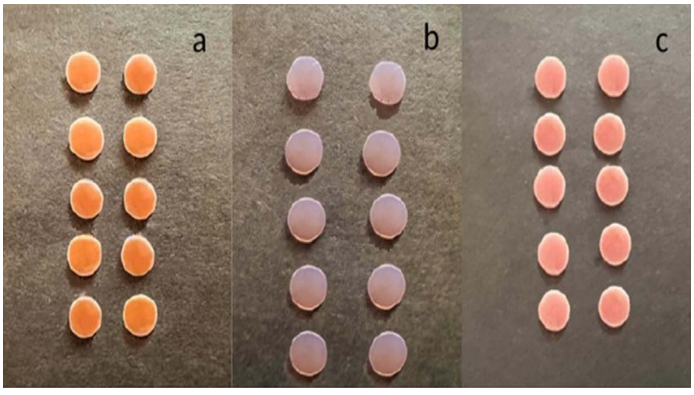

Figure 1. a) Milled specimens, b) 3D printed specimens, c) Heat-polymerized specimens

mm. To facilitate specimen production, this digital design was exported in Standard Tessellation Language (STL) file format. Subsequently, employing an IvoBase CAD system from Ivoclar in conjunction with a five-axis milling machine (HinriMill 5, located in Goslar, Germany), the CAD-CAM specimens were fabricated from a pre-polymerized PMMA resin disc.

The Form 3B+ printer (Formlabs, Somerville, Massachusetts, USA) was utilized to produce the specimens through the use of stereolithography (SLA) 3D printing technology. Commercial use of the Formlabs 3D-printed denture base material is now possible. Each sample had a layer thickness of 50 µm and a construction orientation of 90 degrees. Following printing, the specimens were cleaned for three minutes with 90% isopropyl alcohol using Form Wash, an ultrasonic cleaning device from Formlabs. Then, using FormCure (Formlabs Inc., also based in Somerville, MA, USA), they underwent a 60 minute post-polymerization procedure at 60°C (30 minutes at one temperature followed by another 30 minutes).

To produce specimens using the conventional method, disk-shaped wax samples were initially placed into the flask. Once the gypsum inside the flask had completely set, it was immersed in boiling water to facilitate the melting and removal of the wax. After the wax was eliminated, any negative voids were coated with lacquer, and a heat-polymerized acrylic resin material, Meliodent Heat Cure, was applied into the voids within the gypsum according to the manufacturer's instructions. The flask was then pressed at 100 bars of pressure to remove excess acrylic material and subsequently maintained under 200 bars of pressure for five minutes. The sealed flasks were then placed in cold water and brought to a boil. Once the temperature reached 100°C, the materials were boiled for 20 minutes. The samples were then extracted from the flask and leveled using a precision grinding machine and a hard mill. To simulate the texture of dentures, the specimens were sequentially wet-ground at a speed of 60 rpm using a grinding and polishing machine (Gripo 2V, METKON, Grinder-Polisher) with 400, 600, and 800 grit sandpaper. Measurements were taken systematically using an electronic caliper to ensure uniformity of sample dimensions during grinding and polishing. After sanding, the specimens were polished by the same technician using Ivoclar Vivadent Universal polishing paste and felt materials (22).

The process of thermal cycling

The samples were placed in a bath of distilled water at temperatures between 5°C and 55°C and subjected to 5000 cycles of thermal cycling. Every 60 second cycle had four distinct steps: 20 seconds at 5°C, 10 seconds of transitioning to a different bath, 20 seconds at 55°C, and then 10 seconds of returning to the 5°C bath. 5000 thermal cycles equivalent to approximately six months clinical use (23).

Microbiological evaluation

The samples were initially sterilized using an autoclave. Equal numbers of all samples were placed in sterile petri dishes. An equal amount of prepared yeast suspension was then added to each dish, enough to cover the sample surface. The dishes were then placed on a shaker and incubated for 24 hours at 37°C in a 5% CO2 incubator. For the preparation of the yeast suspension, a suspension was prepared from the standard strain C.albicans ATCC 14053, which was inoculated onto Sabouraud dextrose agar (SDA) agar (BD Difco) and incubated at 37 °C in a 5% CO2 incubator for 24 hours. Subsequently, microorganisms were taken from the growing cultures with sterile loops, and a suspension was prepared to have an absorbance of 0.5 McFarland turbidity standard by measuring absorbance at 530 nm wavelength with a spectrometer. The suspension was further diluted 10 times with Yeast Extract-Peptone-Dextrose (YPD) liquid. These prepared solutions were added to the Petri dishes containing the samples for incubation (24).

After incubation, the samples were gently washed with water and left to dry. Subsequently, they were treated with ethanol for 10 minutes to fixate. Then, they were stained with methylene blue and washed after staining. Once the materials were dry, each material was examined under a microscope (Olympus BX53) at 100X magnification (Figure 2). Ten fields were evaluated for each material, and the numbers of microorganisms in each field were recorded.

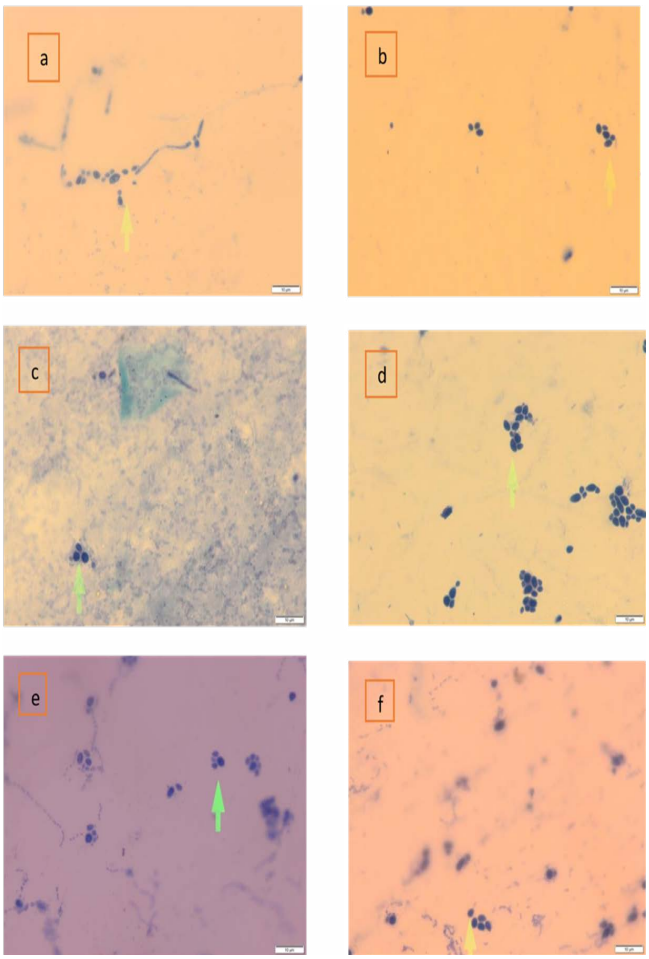

Figure 2. Microscope images; 100 X magnification; a: non aged FL, b: aged FL, c: non aged IB d: aged IB, e: non aged MD, f: aged MD

Table 1. Descriptive statistics (mean ± standard deviations) of C.albicans adhesion

Material	Non-aging	Aging
FL	13,47 ±5,48[b]	10,26 ±3,52[b]
IB	10,05 ±1,60[b]	11,82 ±5,10[b]
MD	38,97 ±11,51[a]	38,77 ±16,99[a]

* Different superscript letters in each column indicates statistically significant differences (p<0.05).

Scanning electron microscopy (SEM)

All specimens in the group were covered with Au and examined under scanning electron microscopy (SEM; SU5000; HITACHI, Japan) at a working distance of 13.8 mm and a voltage of 10 kV. SEM images were analyzed at a magnification of 3000 times.

Statistical analysis

Data were analysed using two-way analysis of variance (ANOVA) using IBM SPSS 20.0 software (SPSS Inc., Chicago, IL). Then, to determine whether there were differences between the groups, a Tukey honest post hoc test was used. Statistical significance was set at $p < 0.05$.

RESULTS

There was no statistically significant difference between the IB and FL groups, whereas Candida adhesion was mostly pronounced in the traditionally manufactured MD group. Candida adhesion was also lower in the next-generation denture base materials. In every tested group, the impact of aging was determined to be statistically insignificant (Table 1).

The type of material used was a significant factor in the results of the two-way ANOVA. However, this significance was reduced when both the type of material and the aging were considered together, and the influence of the aging was found to be negligible (Table 2).

The MD group had deeper scratches, grooves and a more porous surface than the other groups, as shown by the SEM images (Figure 3). For this reason, there was intense candida colonization in MD samples. In comparison, less dense Candida colonisation was observed on the surfaces of the FL and IB, which have a more uniform and smooth surface. The microbial colonies adhered relatively more to the MD.

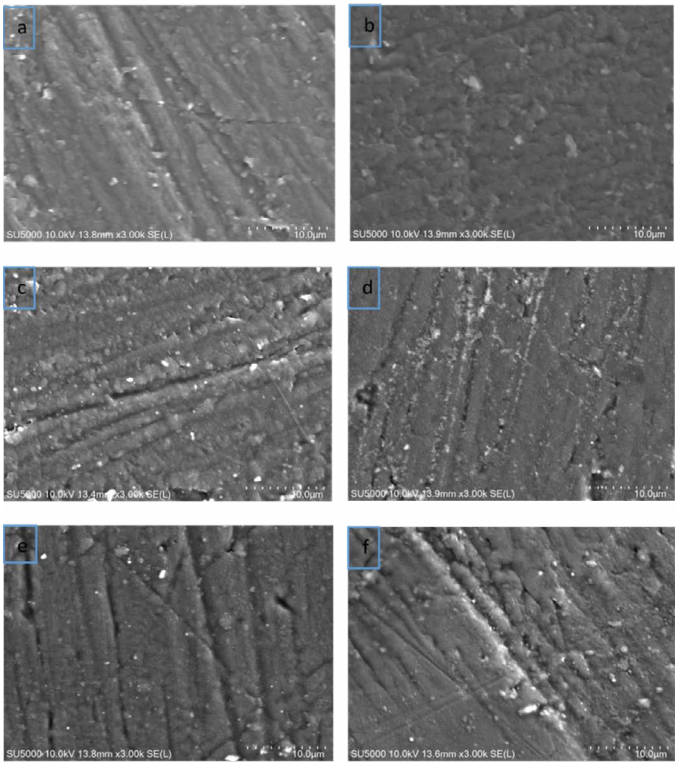

Figure 3. SEM images a: non aged FL, b: aged FL, c: non-aged IB, d: aged IB, e:non-aged MD, f: aged MD

DISCUSSION

This study compared C.albicans adhesion of denture base materials obtained through heat polymerization, CAD/CAM milling, and 3D-printed methods and investigated how the aging process with thermal cycling affected Candida adhesion. The first hypothesis was accepted, as differences were found in Candida adhesion among denture base materials manufactured by different methods. Samples produced with the conventional method showed significantly higher C.albicans adhesion compared to the CAD/CAM milling and 3D-printed methods. The second hypothesis, suggesting an increase in Candida adhesion after thermal cycling in all samples, was rejected, and the impact of thermal cycling was found to be insignificant.

Denture stomatitis is a disease that can affect more than half of the population using removable dentures. Plaque formation on dentures due to inadequate oral hygiene increases microbial colonization, leading to the development of the disease (25). Our study focused on this issue, as C.albicans is identified as

Table 2. Results of two-way ANOVA for C.albicans adhesion

Test method	Source of variation	Sum of squares	df	Mean square	F	p
	Material	10069.994	2	5034.997	160.089	0.06
	Aging	4.483	1	4.483	0.143	0.3742
	Material*Aging	62.902	2	31.451	0.384	0.683
	Error	4427.828	54	81.997		
	Total	25354.593	60			

p<0,05

the primary factor in denture stomatitis, particularly in patients using complete dentures in the upper jaw (26).

In studies comparing heat-polymerized base material with base materials obtained through CAD/CAM milling methods from different brands, it was observed that the amount of C.albicans adhering to specimens produced by CAD/CAM milling was lower than that of heat-polymerized acrylic resin (27-29). Our study also confirmed these results. The degree of polymer conversion in polymer materials affects the remaining monomer levels in the processed material, consequently influencing its physical, mechanical, and microbiological properties (30-32). Higher Candida adhesion in heat-polymerized acrylic resins may be attributed to factors such as monomer evaporation during polymerization, air entrapment during mixing, residual monomer presence, evaporation associated with exothermic reactions, inadequate monomer-polymer mixing, and porosity resulting from insufficient pressure applied to the mold (33). CAD/CAM milled disks are processed under high pressure and temperature, reducing the remaining monomer levels and strengthening the structure and properties of the processed material. These results explain the findings of our study.

Avi et al. (5) conducted a study comparing prosthetic base materials produced with different techniques (heat-polymerized acrylic, cold acrylic, 3D printing method and CAD/CAM milling method) in terms of C.albicans adhesion. They observed that specimens produced with the 3D printing method increased microbial adhesion compared to the conventional method. The discrepancy with our study's findings may stem from variations in the printing method, resin brand differences, and polishing techniques. Similarly, in the same study, the CAD/CAM milling method showed the lowest adhesion, consistent with our study. Likewise, Freitas et al.'s study (34) also showed the CAD/CAM milling method having the lowest Candida adhesion, resembling our findings. However, the 3D-printed group, while not statistically significant, exhibited higher bacterial adhesion than the conventional method. The variation in 3D-printed angle values influencing material properties in previous studies could be considered as a contributing factor. Choosing the printing parameters (printing method, wavelength, film thickness, temperature and final curing time) can affect the results. In a study where the printing method (90, 45, and 0 degrees) did not affect C.albicans adhesion in prosthetic base resins (35), Shim et al. (21) found the highest ratio of C.albicans were present on surfaces printed at 0 degrees, followed by 45 and 90 degrees. Therefore, it was concluded that the 90-degree printing method is more suitable for preventing C.albicans adhesion (36). Li et al (37) evaluated different build angles (90°, 45°, 0°) and print layer thicknesses (100, 50, 25 µm) on the surface properties of prosthetic base resin processed using the DLP additive technique. They found that the adhesion of C.albicans to DLP-printed prosthetic surfaces was influenced by the thickness of the print layer, but not by the build angle. As a consequence of the studies, it was concluded that the thickness of the layer should be less than 100 µm in order to prevent the adhesion of C.albicans (37). Earlier studies by Unkovskiy et al and Altarazi et al achieved the best results in the mechanical and physical features of 3D printed resin using a vertical printing technique (90°) (38-40). With regard to post-curing, Kim et al. (41) suggested a minimum of 60 minutes of UV curing in an oven to develop the physical features of 3D printed parts. The low level of microbial adhesion suggests that the 3D printed resin may be a better option than traditional prosthetic bases. The selection of a 90 degree printing method, a print film thickness of 50 µm and a post cure time of 60 minutes in our study was based on the previous studies.

Fiore et al. (42) confirmed greater adhesion of C.albicans to heat-polymerized PMMA resin within 90 minutes. However, after 16 hours of incubation, the 3D-printed SLA technique and milled prosthetic base resins showed similar microbial adhesion, with all resins showing high microbial adhesion. Arutyunov et al. (43) found differences in the Candida adhesion index among materials produced using the same manufacturing technology. Even the slightest difference in material composition can alter microbial properties (44,45).

Limitations of this study include the complexity and multifactorial nature of microbial adhesion in the oral cavity. It isn't easy to simulate this process in vitro. Further studies are needed on multi-species biofilms and in vivo or situ settings. Additionally, the oral cavity's acquired pellicle affects the surface characteristics and microbial adherence of materials. The main process by which the pellicle is formed is the selective adsorption of salivary proteins, peptides and other macromolecules. Further research is needed under saliva-coating conditions. Various parameters in additive manufacturing can affect the outcomes. For future studies, Candida adhesion results should be evaluated using different printers, various printing parameters, and more brands for each group.

CONCLUSION

Candida infections and associated denture stomatitis are less common in new-generation denture base materials compared to traditional heat-polymerized acrylic. Microbiological evaluation of base materials produced by digital method, which is more comfortable in terms of production technology, was found successful. 3D printed and milled denture base materials produced digitally can be used instead of heat-polymerized acrylics.

The thermal cycle did not affect the adherence of C.albicans in all groups.

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