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Original article

EVALUATION OF CANDIDA SPP. ADHERENCE TO 3D-PRINTED AND HEAT-CURED PMMA RESIN DENTURES

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ABSTRACT

Introduction: Despite the progress of additive manufacturing, there are different opinions regarding microbial adhesion on the surface of 3D printed dentures and the biocompatiability of the materials.

The purpose of this in-vivo study was to determine the presence of Candida species in the saliva, oral mucosa and on dentures of patients wearing heat-cured polymethylmethacrylate (PMMA) and 3D-printed prosthetic appliances after a three-month period of observation.

Materials and methods: The clinical study included 40 edentulous patients, divided into two groups - first group (A) –wearing conventional dentures and second group (B) – wearing 3D printed dentures. It was used chrom agar Candida (Bio Merieux) for identification and quantity assessment of the presence of Candida spp. and spectrophotometric analysis using VITEK MS (Bio Merieux).

Results: After three months of wearing the dentures, Candida spp was not isolated in the saliva in 60% of patients in group A and 70% of group B, with no statistically significant difference between the results before the treatment and after the third month. In the third month, Candida spp was isolated from the oral mucosa of 20% of group A and in group B – 10%, with no significant difference between the groups ($p<0.05$). No Candida spp was isolated from 90% of denture surfaces in both groups.

Conclusion: The adhesion of Candida spp. to 3D printed dentures is not higher than conventional dentures made from heat-curing resin. Also, there is no difference between the amount of Candida spp. in the saliva, oral mucosa and denture surfaces.

Keywords: 3D dentures, Candida spp., denture stomatitis,

INTRODUCTION

Denture stomatitis is a common diagnosis for patients treated with removable dentures. The condition can be localized or generalized, and it is caused by inflammation of the oral mucosa, which is in contact with the denture surface.

The main etiological factor is the presence and increased quantity of Candida species. They are often encountered and considered harmless members of the human microbiome - Candida albicans, Candida tropicalis, Candida glabrata, Candida parapsilosis, Candida stellatoidae, Candida krusei, Candida kefyr and etc. Approximately 200 species of Candida are known, and only 10% of them can cause infections. Among all of them, Candida albicans is most frequently isolated from the oral cavity [1-3].

When the patient is wearing a removable denture, a large part of the oral mucosa is in contact with the denture base, creating suitable conditions for the bacterial and fungal multiplication in the narrow space between the mucosa and the denture. Also, the surface roughness of the materials is considered to predispose colonization.

For many years, the conventional denture fabrication method with heat-curing resin has been utilized and it is well known that the final result mainly depends on the manual skills of the operator and the manufacturing process. However, flaws such as porosity, surface roughness and volumetric changes are common [4, 5].

Polymethylmethacrylate (PMMA) is a primary material used for making removable dentures by conventional and well-known methods. Its qualities, such as low density, good aesthetic characteristics, low cost, easy processing and relatively good physical and mechanical properties, make PMMA the preferred material for the fabrication of different prosthetic and orthodontic appliances [6].

Despite the many advantages of PMMA, its susceptibility to microbial colonization remains a major drawback. Over time, not only bacterial biofilm formation is observed, but microorganisms also have the ability to penetrate into the deeper layers of the polymer [7]. Conversely, the oral cavity is an ideal microbial incubator [8] due to its moisture, moderate temperature, abundance of nutrients [9] and combination of different surfaces [10]. Numerous and diverse microorganisms – bacteria, fungi

and viruses [11] - form a complex ecosystem that influences the oral and the patiens's overall health. If the balance of the ecosystem is disrupted by an increase in the quantity of some of the microorganisms or the immune system is compromised, local and systemic protective reactions of the body are activated [12-14].

It is known that when artificial material is placed within the mouth, its surface quickly becomes colonized by microorganisms [15]. In the presence of dentures, bacterial biofilm forms on their surface [16], which stimulates local inflammatory processes, most commonly manifested as erythema or hyperplasia, known as Denture stomatitis [17].

Cawson noted that Candida spp. are isolated more frequently in cases of denture stomatitis than in healthy oral mucosa, and Candida albicans is the most commonly isolated [18]. This is confirmed by numerous authors over the years [19-21].

The digital revolution and rapid technological development have led to the implementation of new materials and methods for the fabrication of dentures. As a result, the time required has been minimized, as have the possibilities of operator error, and exceptional accuracy is evident in some methods.

In recent years, additive manufacturing has quickly become an alternative to conventional methods for the fabrication of removable dentures.

Currently, conflicting opinions exist in the literature regarding the colonization of microorganisms and the formation of biofilm on the surface of 3D printed dentures, as well as the long-term biocompatibility of the used polymers. According to some authors, denture stomatitis is a problem, even for printed dentures. Their studies show that the colonization of C. albicans to 3D printed materials is even higher than conventional heat-curing resins [22-24].

Based on Arutyunov's research, microbial adhesion to the polymers used for printing dentures is lower, and the risk of denture stomatitis is reduced [25].

Alternatively, Fiore found that there is no difference in the microbial adhesion comparing polymers for 3D printing, milling and heat-curing [26].

PURPOSE

This study aimed to investigate C. albicans adherence to two types of denture base polymers: heat-cured polymethylmethacrylate (PMMA) and 3D-printed.

MATERIALS AND METHODS

The clinical study included 40 completely edentulous patients. The exclusion criteria were:

- Systemic diseases (asthma, diabetes mellitus, Sjogren's syndrome, immunodeficiency states)
- Antibiotic use in the last 3 months
- Radiation and/or chemotherapy in the last 6 months
- Denture stomatitis
- Gingival or periodontal diseases

Patients were divided into two experimental groups:

- First group (group A) – patients treated with conventional full dentures in the upper and lower jaws (n=20)(control group)
- Second group (group B) – patients treated with 3D printed full dentures in the upper and lower jaws (n=20)

Saliva collection (unstimulated) was performed in special sterile containers. The patients were instructed not to eat, smoke or rinse their mouths with antiseptic solutions two hours before sample collection. The biological material was transported to the laboratory in a special bag at a temperature of 4°C.

Laboratory Methods:

All collected materials were plated on chromogenic agar for isolation and identification of Candida spp. The plated materials were cultured for 48 hours at a temperature of 35-37°C under aerobic conditions. Identification of the isolated species Candida was performed using the following methods:

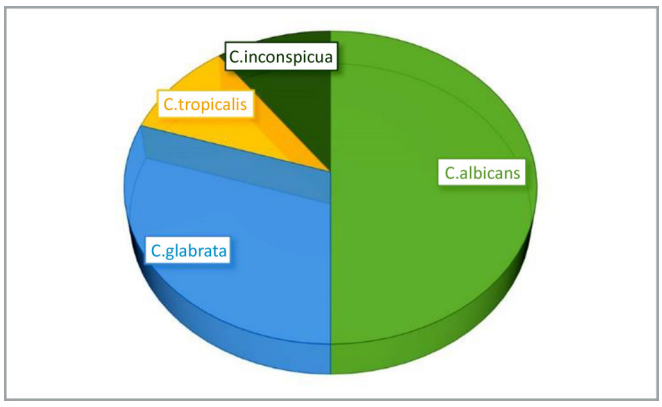
- Direct identification – directly from the chromogenic environment. Indicative of Candida albicans is the characteristic green color.
- For all other Candida species – inoculation onto an individual slide for species identification and performing spectrophotometric analysis using the VITEK MS system.

The identified species Candida are reported as CFU/ml (Colony forming units/ml). Quantities below 10^3 cfu/ml are considered normal, $\geq 10^3$ $<10^4$cfu/ml – low, $\geq 10^{\wedge 4}$ $<10^{\wedge 5}$ cfu/ml are considered moderate and quantities above è $10^{\wedge 5}$ cfu/ml are considered significant.

RESULTS

The results show that before the prosthetic treatment, 50% (20/40) of the patients exhibited no presence of Candida spp. in the saliva and on the oral mucosa. Among the other patients, C. albicans was isolated from 25% (10/40), C. glabrata from 15% (6/40), C. tropicalis from 5% (2/40) and C. inconspicua from 5% (2/40) (Fig.1).

Fig. 1. Qualitative distribution of Candida species.



Of all patients included in the study, 50% (20/40) were wearing old dentures, while the remaining 50% (20/40) had never worn removable denture. Candida spp. were isolated in saliva and on the mucosa in half (10/20) of the patients wearing old dentures. There was no statistically significant difference in the quantity of Candida spp. between the patients with or without dentures ($p<0.05$).

Table 1. The presence of Candida spp. in the saliva.

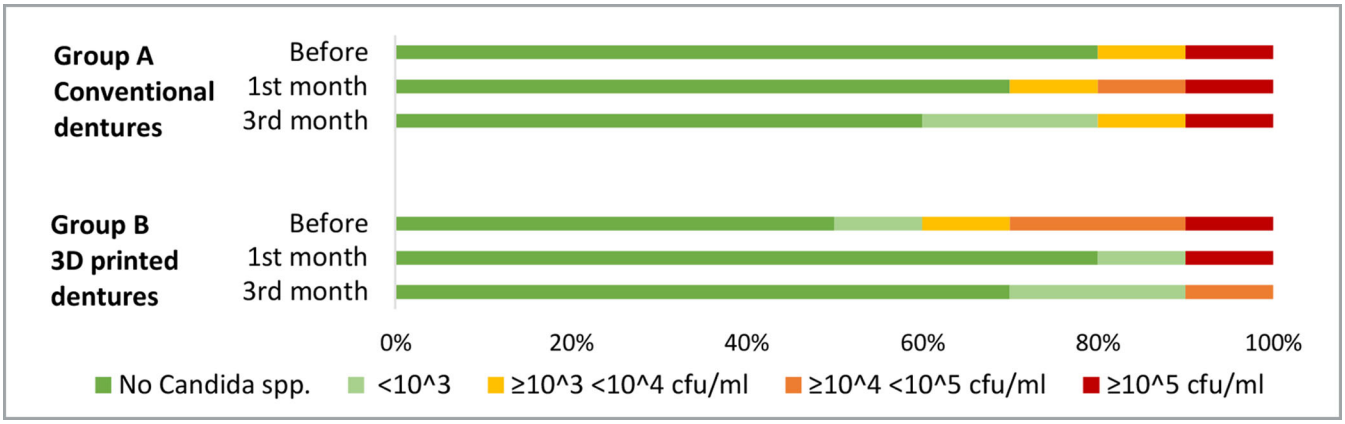
	No Candida spp.		Normal <10^3 cfu/ml		Low ≥10^3 <10^4 cfu/ml		Moderate ≥10^4 <10^5 cfu/ml		Significant ≥10^5 cfu/ml	
	A	B	A	B	A	B	A	B	A	B
Before treatment	80% (16/20)	50% (10/20)	0%	10% (2/20)	10% (2/20)	10% (2/20)	0%	20%	10% (2/20)	10% (2/20)
1 month after treatment	70% (14/20)	80% (16/20)	0%	10% (2/20)	10% (2/20)	0%	10% (2/20)	0%	10% (2/20)	10% (2/20)
3 months after treatment	60% (12/20)	70% (14/20)	20% (4/10)	20% (4/20)	10% (2/20)	0%	0%	10% (2/20)	10% (2/20)	0%

Before denture placement, a statistically significant difference is observed between patients with and without the presence of Candida spp. in saliva only in the control group (A) ($p=0.0293$). Without Candida spp. in the saliva are 80% (16/20) of the patients in group A and 50% (10/20) of group B. In 10% (2/20) of Group B, a normal amount of Candida spp. is observed ($<10^3$ cfu/ml). In 10% (2/20) of both groups, a low amount is found ($\geq 10^3$ $<10^4$cfu/ml). In 20% (4/20) of group B, a moderate amount ($\geq 10^{\wedge 4}$ $<10^{\wedge 5}$ cfu/ml) is observed. In 10% (2/20) of both groups, the quantity is significant ($\geq 10^{\wedge 5}$).

After the first month, the absence of Candida spp. in saliva was observed in 70% (14/20) of patients in group A and 80% (16/20) of patients in group B. No statistically significant difference is found between the results before the treatment and after one month of wearing the dentures (Fig. 2).

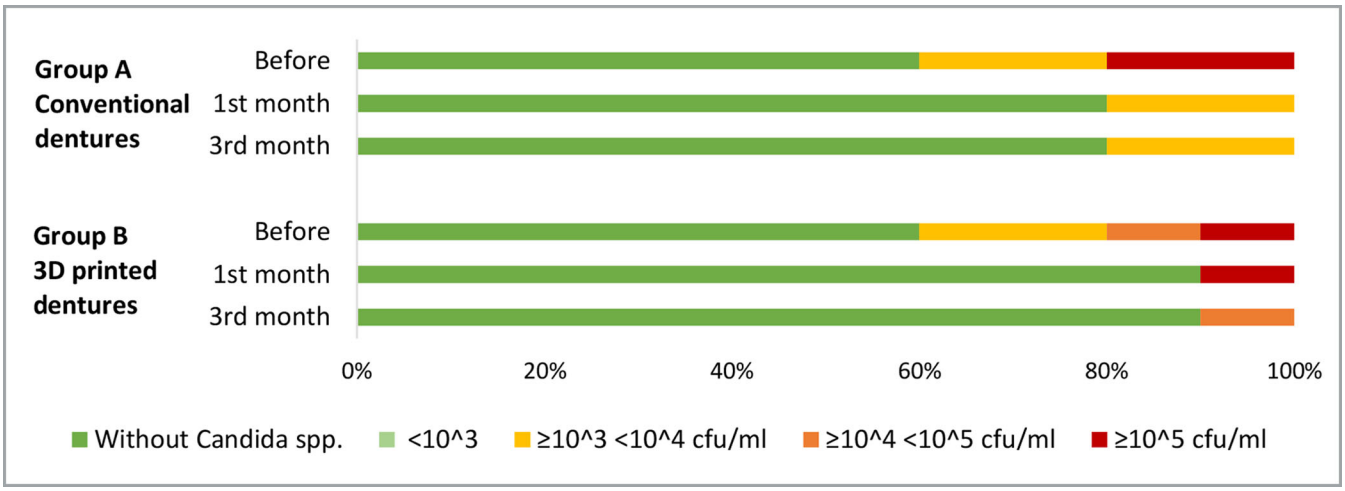
Three months after denture placement, the absence of Candida spp. in saliva is observed in 60% (12/20) of group A and 70% (14/20) of group B, again with no statistically significant difference between those results and before the treatment. In group A, 20% (4/20) of patients have a presence of Candida spp. in saliva, which is considered normal, 10% (2/20) have a low quantity, and 10% (2/20) have a significant quantity. In group B, 20% (4/20) of patients have a presence of Candida spp. in saliva, which is within the normal range, and 10% (2/20) show a moderate quantity. No significant difference is found between the two groups ($p<0.05$).

Fig. 2. The quantity of Candida spp. in the saliva.



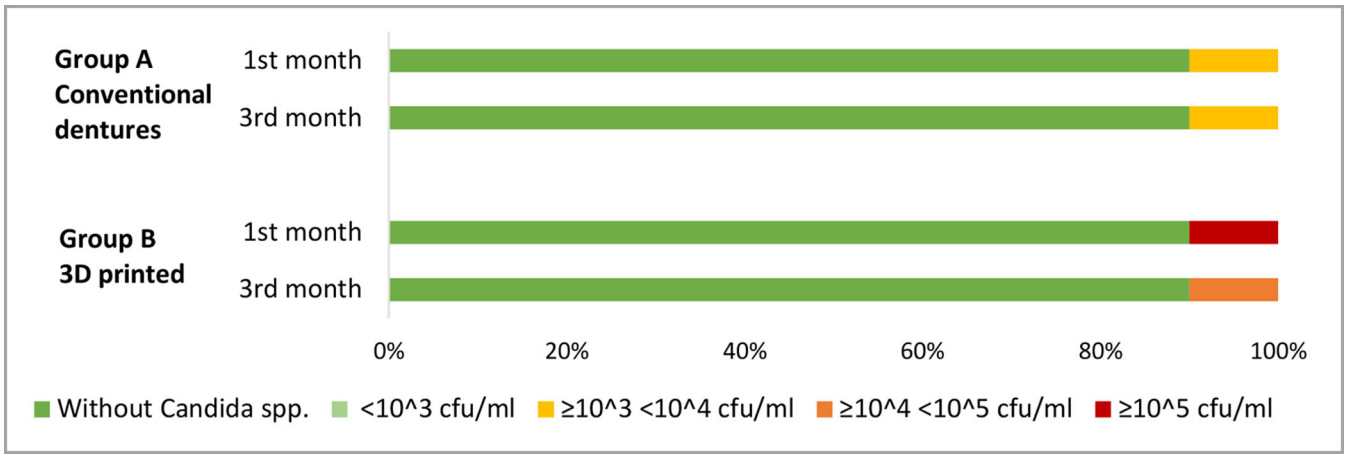
Before the treatment in both groups, the presence of Candida spp. on the oral mucosa was observed in 40% (8/20) of patients (Fig. 3). In the third month in group A, there is a decrease and in 20% (4/20) a low quantity of Candida spp. is isolated. There is also a decrease in group B, and in 10% (2/20) Candida spp. is isolated in moderate quantity. However, there is no statistically significant difference between the results of the two groups ($p<0.05$).

Fig. 3. Quantity of Candida spp. in the oral mucosa.



Regarding the Candida spp. adhesion to denture bases, the results show that on the heat-curing resin, only 10% (2/20) of a small quantity of Candida spp. is isolated in the first and third month. On the surfaces of 3D printed dentures, a significant quantity of Candida spp. is observed in 10% (2/20) of the patients after 1 month, while the quantity is moderate in the third month. Free of Candida spp. are 90% of the denture surfaces in both groups, signifying that the choice of material for removable complete denture has minimal impact on colonization (Fig. 4).

Fig. 4. Quantity of Candida spp. on the denture surface.



DISCUSSION

Considering the recent introduction of additive technology and its implementation in the production of complete dentures, there is a lack of clinical studies demonstrating the biocompatibility of the materials. Exploring the microbial adhesion to denture surfaces, saliva and oral mucosa in patients treated with 3D printed dentures aims to verify the biological tolerance of the used polymers as well as to compare them with the conventional heat-curing resins.

The results from our study validate the findings of Fiore's previous research [26] that the surface of 3D printed denture is not colonized by more quantity Candida spp. compared to conventional dentures made from heat-curing resin. Also, the risk of denture stomatitis is not higher compared to the other manufacturing methods.

However, there are conflicting opinions regarding the biocompatibility of the polymers used for 3D printing and whether the risk of denture stomatitis is higher for patients treated with them in comparison with conventional or milled dentures. According to some authors, the colonization of Candida spp. tends to be greater on the polymers utilized in 3D printing, owing to the additive process and the layered composition of the denture base and teeth. This encourages modification of the materials and efforts aimed at formulating antibacterial polymers suitable for additive technology.

According to Silva MDDD, et al. [22], 3D printed dentures are more susceptible to microbial colonization due to the higher surface free energy of the polymers. Osman RB, et al. [27] highlights that patients wearing printed dentures are predisposed to denture stomatitis as-

sociated with increased levels of Candida spp., emphasizing the importance of oral hygiene.

Arutyunov S, et al. [25] compared the microbial adhesion to four types of resins – cold-cured, heat-cured, milled and 3D printed, observing the lowest values in the group of 3D printed samples. Fiore AD, et al. [26], in his investigation on the colonization of S.mutans, L.salivarius and C.albicans, found that the lowest values were recorded on the 3D printed samples. However, there was no statistically significant difference between all of the groups.

Some authors note [19, 28] that the quantity of C.albicans attached to the denture surface is significantly higher than that found on the oral musca, which corresponds with the findings we observed.

In terms of qualitative traits of the isolated species, our findings indicate that C.albicans is detected in 50% of the cases, followed by C.glabrata, C.tropicalis and C.inconspicua. Herman P, et al. [29] extensively examines oral candidiasis and its causative factors, confirming our findings.

Mousa MA, et al. [30] also found that the most commonly isolated species in patients wearing removable dentures are C.albicans, followed by C.glabrata. They observed that colonization on the denture surface begins immediately after inserting the denture in the oral cavity, with no significant difference in the quantity of Candida spp. between the first and the second month, which aligns with our results showing no significant difference between the first and the third month post-treatment.

CONCLUSION

The conclusions from the conducted clinical study are as follows:

• Colonization of Candida spp. on dentures fabricated using additive technology is not higher than that observed with conventional dentures made of heat-curing resin.

• The manufacturing method does not affect the risk of denture stomatitis in patients treated with removable dentures

• There is no statistically significant difference in the amount of Candida spp. colonizing the saliva, oral mucosa and denture surfaces

• There is no statistically significant difference in the amount of Candida spp. between the first and the third month after the denture placement

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REFERENCES:

1. Yigit N, Aktas E, Dagistan S, Ayyildiz A. Investigating biofilm production, coagulase and hemolytic activity in Candida species isolated from denture stomatitis patients. *Eurasian J Med.* 2011 Apr;43(1):27-32. [PubMed]

2. Kinkela Devcic M, Simonic-Kocijan S, Prpic J, Paskovic I, Cabov T, Kovac Z, et al. Oral Candidal Colonization in Patients with Different Prosthetic Appliances. *J Fungi (Basel).* 2021 Aug 16;7(8):662. [PubMed]

3. Lewis MAO, Williams DW. Diagnosis and management of oral candidosis. *Br Dent J.* 2017 Nov 10;223(9):675-681. [PubMed]

4. Wong DM, Cheng LY, Chow TW, Clark RK. Effect of processing method on the dimensional accuracy and water sorption of acrylic resin dentures. *J Prosthet Dent.* 1999 Mar;81(3):300-4. [PubMed]

5. Zissis AJ, Polyzois GL, Yannikakis SA, Harrison A. Roughness of denture materials: A comparative study. *Int J Prosthodont.* 2000 Mar-Apr;13(2):136-40. [PubMed]

6. Zafar MS. Prosthodontic Applications of Polymethyl Methacrylate (PMMA): An Update. *Polymers (Basel).* 2020 Oct 8;12(10):2299. [PubMed]

7. Takeuchi Y, Nakajo K, Sato T, Koyama S, Sasaki K, Takahashi N. Quantification and identification of bacteria in acrylic resin dentures and dento-maxillary obturator-prostheses. *Am J Dent.* 2012 Jun;25(3):171-5. [PubMed]

8. Yankova M, Yordanov B, Baykuchev R, Raykova V, Mitov I. Presence of Candida spp. in the saliva of patients with complete dentures, lined with silicone-based elastic materials. *J of IMAB.* 2017 Oct-Dec;23(4):1813-1822. [Crossref]

9. Hao Y, Huang X, Zhou X, Li M, Ren B, Peng X, et al. Influence of Dental Prosthesis and Restorative Materials Interface on Oral Biofilms. *Int J Mol Sci.* 2018 Oct 14;19(10):3157. [PubMed]

10.Contaldo M, Romano A, Mascitti M, Fiori F, Della Vella F, Serpico R, et al. Association between denture stomatitis, candida species and diabetic status. *J Biol Regul Homeost Agents.* 2019 May-Jun;33(3 Suppl. 1):35-41. [PubMed]

11.Mosaddad SA, Tahmasebi E, Yazdanian A, Rezvani MB, Seifalian A, Yazdanian M, et al. Oral microbial biofilms: an update. *Eur J Clin Microbiol Infect Dis.* 2019 Nov;38(11):2005-2019. [PubMed]

12.Zhang Y, Wang X, Li H, Ni C, Du Z, Yan F. Human oral microbiota and its modulation for oral health. *Biomed Pharmacother.* 2018 Mar;99:883-893. [PubMed]

13. Sampaio-Maia B, Caldas IM, Pereira ML, Pérez-Mongiovi D, Araujo R. The Oral Microbiome in Health and Its Implication in Oral and Systemic Diseases. *Adv Appl Microbiol.* 2016;97:171-210. [PubMed]

14. Arweiler NB, Netuschil L. The Oral Microbiota. *Adv Exp Med Biol.* 2016; 902:45-60. [PubMed]

15. Busscher HJ, Rinastiti M, Siswomihardjo W, van der Mei HC. Biofilm formation on dental restorative and implant materials. *J Dent Res.* 2010 Jul;89(7):657-65.[PubMed]

16. Schmalz G, Cieplik F. Biofilms on Restorative Materials. *Monogr Oral Sci.* 2021; 29:155-194. [PubMed]

17. Hannah VE, O'Donnell L, Robertson D, Ramage G. Denture Stomatitis: Causes, Cures and Prevention. *Prim Dent J.* 2017 Dec 1;6(4): 46-51. [PubMed]

18.Cawson RA. Symposium on denture sore mouth. II. The role of Candida. *Dent Pract Dent Rec.* 1965 Dec;16(4):138-42. [PubMed]

19.Budtz-Jörgensen E, Stenderup A, Grabowski M. An epidemiologic study of yeasts in elderly denture wearers. *Community Dent Oral Epidemiol.* 1975 May;3(3):115-9. [PubMed]

20.Gleiznys A, Zdanavièienë E, Þilinskas J. Candida albicans importance to denture wearers. A literature review. *Stomatologija.* 2015; 17(2): 54-66. [PubMed]

21.Kalberer N, Mehl A, Schimmel M, Müller F, Srinivasan M. CAD-CAM milled versus rapidly prototyped (3D-printed) complete dentures: An in vitro evaluation of trueness. *J Prosthet Dent.* 2019 Apr; 121:637-43. [PubMed]

22.Silva MDDD, Nunes TSBS, Viotto HEDC, Coelho SRG, Souza RF, Pero AC. Microbial adhesion and biofilm formation by Candida albicans on 3D-printed denture base resins. *PLoS One.* 2023 Oct 4;18(10): e0292430. [PubMed]

23.Koujan A, Aggarwal H, Chen PH, Li Z, Givan DA, Zhang P, et al. Evaluation of Candida albicans Adherence to CAD-CAM Milled, 3D-Printed, and Heat-Cured PMMA Resin and Efficacy of Different Disinfection Techniques: An In Vitro Study. *J Prosthodont.* 2023 Jul;32(6): 512-518. [PubMed]

24.Meirowitz A, Rahmanov A, Shlomo E, Zelikman H, Dolev E, Sterer N. Effect of Denture Base Fabrication Technique on *Candida albicans* Adhesion In Vitro. *Materials (Basel).* 2021 Jan 5;14(1):221. [PubMed]

25.Arutyunov S, Kirakosyan L, Dubova L, Kharakh Y, Malginov N, Akhmedov G, et al. Microbial Adhesion to Dental Polymers for Conventional, Computer-Aided Subtractive and Additive Manufacturing: A Comparative in Vitro Study. *J Funct Biomater.* 2022 Apr 11;13(2):42. [PubMed]

26.Fiore AD, Meneghello R, Brun P, Rosso S, Gattazzo A, Stellini E, et al. Comparison of the flexural and surface properties of milled, 3D-printed, and heat polymerized PMMA resins for denture bases: An in vitro study. *J Prosthodont Res.* 2022 Jul 30;66(3):502-508. [PubMed]

27.Îsman RB, Khoder G, Fayed B, Kedia RA, Elkareimi Y, Alharbi N. Influence of Fabrication Technique on Adhesion and Biofilm Formation of *Candida albicans* to Conventional, Milled, and 3D-Printed Denture Base Resin Materials: A Comparative In Vitro Study. *Polymers (Basel).* 2023 Apr 10;15(8):1836. [PubMed]

28. Nanetti A, Stancari F, Ferri M, Mazzoni A. Relationship between Candida albicans and denture stomatitis: a clinical and microbiological study. *New Microbiol.* 1993 Jul; 16(3):287-91. [PubMed]

29.Hermann P, Berek Z, Nagy G, Kamotsay K, Rozgonyi F. Pathogenesis, microbiological and clinical aspects of oral candidiasis (candidosis). *Acta Microbiol Immunol Hung.* 2001; 48(3-4):479-95. [PubMed]

30. Mousa MA, Lynch E, Kielbassa AM. Denture-related stomatitis in new complete denture wearers and its association with Candida species colonization: a prospective case-series. *Quintessence Int.* 2020; 51(7): 554-565. [PubMed]

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