Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

The impact of different occlusal guard materials on *Candida albicans* proliferation in the oral cavity

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Article Info

Article history:

Received: August 02, 2024
Accepted: November 04, 2024
Published: November 30, 2024

Use your device to scan and read the article online

Abstract

Candida albicans is an opportunistic fungal pathogen. It's a dimorphic fungus with hyphal form that can penetrate and proliferate the oral mucosa. Occlusal guard materials come into direct contact with the oral mucosa and saliva when worn for extended periods, the occlusal guard acts as a reservoir for *C. albicans* that imposes adverse oral or systemic effects, particularly in medically compromised patients. A randomized controlled trial was conducted among forty volunteers with a history of bruxism. The volunteers were divided into four groups, with each group assigned to wear occlusal guards made of one of the following materials: (Polyethylene Terephthalate-Glycol, Polymethyl methacrylate resin, Ethyl phenylphosphinate 3D printing resin and Chrome-Cobalt Alloy). The study samples were collected after one month, with an additional three months spent assessing *C. albicans*. A descriptive statistical analysis was performed and compared between groups with different time intervals. The statistical analysis revealed that *C. albicans* proliferation increased after three months of wearing the occlusal guards, however, the results showed non-significant differences (P = 0.914). Furthermore, the comparative analysis demonstrated that the highest proliferation of *C. albicans* was found with Polymethyl methacrylate and the least with Chrome-Cobalt Alloy. Within the limitations of this study, it was concluded that reducing wearing time will reduce pathogenic infection by *C. albicans,* and the occlusal guard with the chrome-cobalt alloy material was better than the other materials in this aspect.

Keywords: Bruxism, *C. albicans*, Occlusal guard materials, Chrome-Cobalt alloy, Ethyl phenylphosphinate 3D printing resin, Polyethylene Terephthalate-Glycol Copolyester, Polymethyl methacrylate resin

1. Introduction

The evaluation of various dental materials that are used for fabricating prosthetic appliances is no longer exclusively dependent on the mechanical and physical characteristics of the material, but also on its microbial properties [1]. *C. albicans* is an opportunistic fungal pathogen. It's a dimorphic fungus with hyphal form that can penetrate and proliferate the oral mucosa [2]. *C. albicans* is a resident microflora and may be isolated in 30–65% of healthy people [3]. The adhesion of microorganisms to biomedical surfaces is primarily influenced by composition of the material, and electrostatic interactions [4]. Hydrophobicity, surface free energy [5–7], and surface roughness [8]. Furthermore, the manufacturing process of the material had an effect on the surface topography such as porosities, surface defects, and microbiological properties [9, 10].

Microbial species growth on occlusal guards and other prosthetic materials can cause a numerous issue, including bad breath, dental plaque, material pigmentation and staining, and calculus deposits that promote the attachment of oral microorganisms and related systemic and oral diseases like *C. albicans* [11], which is considered one of the main causes of several oral diseases such as oral stomatitis, caries or radicular decay, candidiasis, gingivitis, and periodontitis [12].

Occlusal guard materials come into direct contact with the oral mucosa and saliva. Consequently, when worn for extended periods, these guards can act as a reservoir for *C. albicans*, potentially leading to adverse oral or systemic effects, particularly in medically compromised patients [13]. Therefore, it is essential to investigate the impact of different occlusal guard materials on *C. albicans* proliferation in the oral cavity. This research aims to identify materials with the least microbial adherence to occlusal guards, aiding in the selection of materials that mitigate *Candida*-related complications.

2. Materials and Methods

A longitudinal study was performed for forty volunteers wearing maxillary stabilized occlusal guards (20 males and 20 females), with age groups from 25-45 years old. The diagnosis was established by using a comprehensive case history, intraoral examination, and clinical evaluation [14]. After receiving explanations about research and ex-

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Doi: http://dx.doi.org/10.14715/cmb/2024.70.11.18

pressing their consent through signed terms of agreement and informed consent, the volunteers were allowed to take free participate in the study.

The inclusion criterion was patient complaints with signs and symptoms of myofascial pain and good general health [15], with class I occlusal relationship, While the excluded criterion included those with recent facial trauma, pregnancy, major malocclusion, dental pain, previous treatment for TMDs. Dental therapy including orthodontic treatment; as well as those with more than two missing posterior teeth excluding third molars or use of removable prosthesis. Those with systemic conditions (eg, fibromyalgia, osteoarthritis), major neurological or psychiatric disorders, and the ongoing use of medication, such as benzodiazepines, antipsychotics, or antidepressants were also excluded from the study [15, 16].

The volunteers were divided into four groups; each group consisting of 10 patients. Occlusal guards of each group were constructed with different types of material including; Hard transparent thermoplastic sheets of Polyethylene Terephthalate-Glycol Copolyester (PET-G) by Vacuum forming technique with Biostar pressure molding machine, 1.5 mm thickness bond to self-curing resin (LOT no. 4820A: Shade; Clear; Scheu Duran, Tonawanda, NY 14150 United States) [17, 18]; Transparent auto-polymerizing polymethylmethacrylate (PMMA) material by Compression molding technique (LOT no. XY071P01; Vertex-Dental, 3705HJ Zeist The Nethorlands) [6, 16]; Light-induced polymerization of liquid resin monomers of Ethyl (2,4,6-trimethylbenzoyl) phenylphosphinate (TPO-L) by additive three-dimensional (3D) digital printing technique (LOT no. 250813; Shade: Clear; Carl-Zeiss-Straße 4, 76275 Ettlingen; Germany) [6, 19, 20]; and Chromium-cobalt (Cr-Co) occlusal guard by investment casting technique (LOT no. 13879; BEGO; Bremen, Germany).

The patients were instructed to wear occlusal guards while sleeping for 8-10 hours for three months for treatment of orofacial pain. Additionally, they were advised to clean their occlusal guards by using a soft toothbrush, and lukewarm water, then rinse well immediately, only after removing them in the morning, and to keep it in water when not in use to prevent dimensional changes [16].

The purpose of the study was to compare the proliferation of *C. albicans* on the different types of occlusal guard materials in order to determine the pathogenicity of *C. albicans* susceptibility to occlusal guard.

2.1. Isolation of *C. albicans*

The *C. albicans* proliferation on the occlusal guards after one- and three- months of wearing the appliances by using the following method; the patients were instructed not to remove the occlusal guard or eat anything on the day of the examination [21], the occlusal guards were taken from the patients (still wear it) in the morning by the researcher, then soaked in 20 ml of Broth containing in sterile closed screw cap containers for approximately 12 hours with interval shaking.

Typically, samples were processed for microbiological analysis by culturing them under standard aseptic conditions in sabouraud dextrose agar medium (LOT no. SDA-2106; Microxpress®, Verna, Goa - 403 722, INDIA) used for culture of yeast microorganism. The tubes were harvested by centrifuging them for 20 minutes at 2300 rpm and discarding the supernatant. The resultant deposit of tubes was then resuspended in 500 µl of sterile saline and well-shaken using a mechanical vortex mixer. Next, pipetted 100 µl of concentrate and immediately inoculated [5], onto SDA plates with streak technique, then SDA plates were incubated aerobically at 37°C for 24-48 hours and under suitable situations for the microorganism, for quantification of *C. albicans*, then the colonies were counted, count each colony dot once [22].

Microbial quantity of adherent *C. albicans species* to the occlusal guard in oral cavity was calculated by the number of colonies stated as colony-forming units per millimeter by corresponding dilution factor (cfu/ml = number of colonies x dilution factor) were counted per volume of culture agar plate [5]. According to Brugger, et al the suitable colony counting range is 25-250 colonies per plate, this range also varies according to the method used. When the plates were counted with too many colonies (overgrowth) or more than 250 CFUs/mL the samples would be prepared by serial dilution. On the other hand, plates with less than 25 colonies do not have a statistically significant number of colonies [22]. When the plates were counted with a small number of colonies, the time was increased for incubation (could increase even to 7 days). In this study, each time of serial dilution, 100 µl of concentrate was diluted in 900 µl of sterile saline.

2.2. Statistical analysis

The data were entered into Microsoft Excel sheet, then transferred to SPSS version 27 (IBM, 2020) for analysis. Numerical data were explained by mean (standard deviation) or median (IQR: interquartile range). Categorical data were described by frequency and frequency percentages. As the data were non-normal (skewed to the right), Mann-Whitney and Kruskal-Wallis tests (nonparametric statistical tests) were used to analyze numerical data. Chi-square test or (when it was inappropriate due to low frequency) Fisher-Freeman-Halton exact test was applied to categorical data analysis. A P value of less than 0.05 was regarded as statistically significant load by the Fisher-Freeman-Halton exact test and Wilcoxon signed ranks test.

3. Results

The proliferation of *Candida* species (CFU/mL) in the oral cavity of individual worn occlusal guards, was evaluated in relation to different types of occlusal guard materials after one- and three-months load by Fisher-Freeman-Halton exact test and Wilcoxon signed ranks test. Statistically non-significant differences were demonstrated between all tested materials (Fig. 1 and Table 1). However, the comparative analysis of results, presented as percentages of CFU/mL after one month and three months in the study, revealed the greatest proliferation of *Candida species* on PMMA occlusal guard material, while the lowest was observed on Cr-Co occlusal guard material.

In general, the oral *C. albicans* colonization increased with time of wearing occlusal guards, in all tested materials, however, the result was statistically non-significant differences (P= 0.914), where statistically significant (P< 0.05) as represented in Table 2.

4. Discussion

The normal physiological oral microbiota components include *Candida* species and predominantly *C. albicans* [16] and are found in 50-60% of the population. Microor-

Table 1. The percentages Comparison of total count of oral *C. albicans* colonization (CFU/mL) categories by time among different occlusal guard materials.

Material of occlusal guard	Time	CFU/mL categories							P value*
		<25		25-250		>250		Total	
		No.	%	No.	%	No.	%	No.	
PET-G	1 month	5	50.0	2	20.0	3	30.0	10	0.700
	3 months	7	70.0	1	10.0	2	20.0	10	
PMMA 1	1 month	5	50.0	2	20.0	3	30.0	10	1.000
	3 months	4	40.0	2	20.0	4	40.0	10	
TPO-L	1 month	5	50.0	3	30.0	2	20.0	10	1.000
	3 months	5	50.0	3	30.0	2	20.0	10	
Cr-Co	1 month	7	70.0	1	10.0	2	20.0	10	1.000
	3 months	6	60.0	2	20.0	2	20.0	10	

* Based on Fisher-Freeman-Halton exact test.

Table 2. Comparison of overall *C. albicans* Colonization (CFU/mL) after one month and after three months.

CFU/mL (*Candida species*)	No.	Mean	Median	IQR	P value*
after 1 month	40	12293	4	288	0.914
after 3 months	40	173491	10	289	

* Based on Wilcoxon signed ranks test.

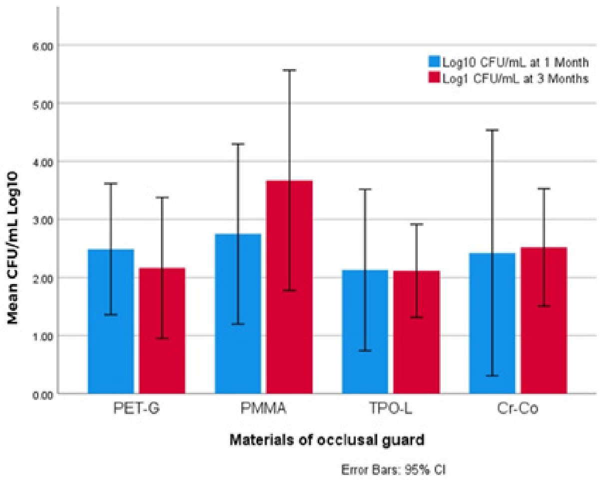

Fig. 1. Mean and standard deviation of CFU/mL (Log10) obtained in different occlusal guard materials after one- and three months of use. (Cr-Co) Chrome-Cobalt alloy. (PET-G) Polyethylenterephthalat-Glycol Copolyester binds to self-curing resin. (PMMA) Polymethyl methacrylate resin. (TPO-L) Ethyl phenylphosphinate 3D printing resin.

ganisms can proliferate due to a variety of factors, and one significant reason is the establishment of habitats that provide favorable conditions for the growth and survival of *Candida species* [23]. Variables that determine the virulence of *Candida species* can be unchained by stimuli from local environment including gaseous compensation, pH, and nutritional availability [24].

Significant variations in fungal adhesion were observed by several authors between prosthetic base resin materials that were polished and roughened [25, 26]. Actually, in clinical settings, the fitting surfaces of maxillary prostheses are never polished or finished, in order to preserve the retention and fitness of the fabricated appliance [10]. The surface characteristics of the manufactured part are influenced by the manufacturing process, which in turn affects the material's microbiological characteristics [27, 28].

As material science has advanced, numerous prosthetic materials such as metals, resins, and, more recently, flexible prosthetic base materials have been utilized over the years. Upon as 90% of denture stomatitis cases have been linked to *C. albicans* adherence to different prosthetic base materials [29].

In general, this study shows that oral *Candida* colonization increased with time of wearing occlusal guards in all tested materials, however, the result did not reveal any significant variations. Furthermore, the occlusal guard from conventional compression of cold-cure PMMA material was non-significance increasing the vulnerability of occlusal guard resins to the adhesion of *C. albicans* in comparison to the additive 3D printing technology of polymer materials and Vacuum forming technique for thermoplastic resin. Whereas the cobalt–chromium alloy material with a lower rate of *Candida* growth when compared to other study resin materials. This result is consistent with the findings of other studies that report that individuals using acrylic resin-based material showed significantly more frequent colonization of *Candida species* when compared with those using metal-based material, and they connected these variations with specific features of the dental materials under investigation [8]. As stated Darwish and Eguia et al., compared the colonization of *Candida* in patients wearing metal and acrylic prostheses, and they concluded that the material properties mentioned could influence on the formation of biofilms. A Significantly higher *Candida* growth was observed when comparing PMMA-based materials to metal alloys [21, 30]. A number of parameters have already been discovered to affect *Candida species*' adherence to acrylic surfaces, such as salivary pellicle, surface roughness, and hydrophobic and electrostatic interactions, even if the precise mechanisms underlying this process are unknown [7].

Our results were also in agreement with another study by Paliwal and Dadarwal, which evaluated and compared *C. albicans* adhesion on the three most popular prosthetic materials: cobalt-chromium alloy, flexible denture base resin, and heat-cured acrylic prosthetic base resin. Flexible prosthetic base material had the greatest *C. albicans* attachment, followed by heat-cured acrylic resin, and the removable prosthetic material made of cobalt-chromium alloy had the lowest amount of *Candidal* attachment. The study findings suggest that the metallic removable prosthetic material has a much lower incidence of prosthetic stomatitis caused by *candidal* infection than acrylic removable prosthetic material. Additionally, they appear to improve biocompatibility of oral tissues, exhibiting little tissue reactions if any. Furthermore, the rigid heat cure acrylic performed better than flexible prosthetic bases, the study concluded that colony-forming units which were observed in a cured and unfinished state, might account for differences in the results as flexible prosthetic material is more difficult to polish and finish due to its fiber content. Metallic base materials are thought to be preferable in numerous respects and have the advantage of having smooth, impermeable hydrophobic surfaces compared to acrylic [31]. Buergers et al. and Celik et al. discovered similar results that the acrylic resin base material had more *candida* adherents than the Co-Cr alloy base material, as a result, the individuals wearing partial dentures with Co-Cr metallic frameworks had a higher frequency of red oral lesions. This is especially true when these appliances are unexpectedly worn for long periods of time in the oral cavity. These findings showed that *C. albicans*' adhesion and, consequently, the prevalence of *candida* stomatitis, are significantly influenced by the type of prosthetic base material [32, 33].

The study conducted by Kinkela Devcic et al. intended for individuals utilizing various prosthetic appliances and found that dentures constructed with PMMA raise the risk of *Candida* colonization, with *C. albicans* being the most predominant species. Additionally, there was a significant decrease in the salivary flow rates of those patients, which contributed to the development of *Candida* colonies. Measurements of salivary flow rate were better in individuals wearing metal framework-based dentures, and they also developed *Candida* colonies less frequently [5].

Metallic materials such as Co-Cr alloys, Ti alloys, stainless steel, etc, are some of the most important materials for engineering that are frequently used as biomaterials because of their high mechanical properties, biocompatibility, and thermal conductivity [34]. In comparison to PMMA-based materials, devices with cast metal bases made of Co-Cr alloys are lighter, more comfortable, stronger, thinner, and smoother on the surface. They also have a lower tendency for surface porosity formation. Consequently, when fewer stable materials are used, the conditions are ideal for *Candida* to multiply unhindered in the small space between the mucosa and appliances [3]. A recognized risk factor for the growth and spread of *Candida*, which leads to the clinically evident oral infection. An easier way to prevent infections and reduce their frequency is to comprehend the causes of development of diseases [12].

Özarslan, et al. investigate the adherence of *C. albicans* to occlusal appliances made using various materials and techniques: vacuum-formed thermoplastic resin, conventional fabrication thermosetting polymethylmethacrylate, computer-aided design and computer-aided manufacture CAD-CAM and 3D printed resin. Following a variety of finishing techniques, the specimens' surface characteristics were assessed using surface free energy, surface roughness measurements, elemental and topographic analysis, and an assessment of biofilm formation alone and in combination with antifungal agents, which is clinically important. The in vitro study's results led to the following conclusions: When compared to conventional manufacturing, The technologies of 3D printing and CAD-CAM enhanced the materials' vulnerability to the adherence of *C. albicans*. Surface roughness values in conventional fabrication and 3-dimensionally printed were significantly higher than in other occlusal device materials. While the greatest surface free energy measurements were in vacuum-formed thermoplastic and computer-aided design and computer-aided manufacture (CAD-CAM). Eugenol was an efficient antibiofilm agent for device materials [35].

In the current study, regarding resin materials of conventional design; thermoplastic sheets (PET-G) of occlusal guard showed the lowest *Candida* proliferation compared to auto-polymerizing polymethylmethacrylate (PMMA) and (TPO-L) by additive 3D printing. This finding was consistent with a previous study by Patil et al. indicating that thermoplastic resin sheets which are used in a variety of clinical scenarios, because of their flexibility and easy adaptation showed a significant reduction in *Candida albican* adherence in comparison to heat-cure and self-cure PMMA resins. Surface irregularities that give the surface roughness can act as a reservoir, improve the retention of microorganisms, and protect them from shear stresses [36].

Similar research by Ahmad et al. demonstrated that compared to acrylic resin denture base materials, *C. albicans* had fewer opportunities to adhere to Valplast [37]. He et al. reported that a comparative study between heat- and cold-cured polymers showed that the microorganisms *C. albicans*, *Candida glabrata*, and *Candida krusei* adhered more strongly to the cold-cured materials [38].

Additionally, our results were in contrast to study done by Schubert, et al. showed that modern CAD/CAM technologies, milling and 3D printing occlusal guard resins, increase the sensitivity to *C. albicans*' adhesion compared to conventional compression molding technique. Additional in vitro and in vivo research is necessary to validate these findings, since they may have clinical significance for people with compromised health who may develop oral or systemic candidiasis [6]. Similar to other studies when evaluating the microbial response to base resin prosthetic materials made with CAD/CAM milling, 3D printing technologies, as well as conventional flask compression molding technique, it was found that the biofilm formation and *candida* adhesion were significantly higher on the 3D-printed specimens. In additive 3D printing technology, microscopic indentations and porosities appear at the intersection of the layers created during the fabrication process. The substantially higher *candida* adhesion seen in the 3D-printed specimen is considered to be the result of this roughness and development of *candida*-associated denture stomatitis [6, 10]. Field Emission Scanning Electron Microscopy (FESEM) optical analysis indicates the roughest surface topography for unpolished 3D-printed specimens as compared with their milled CAD/CAM PMMA, the smoothest surface topography, and the lower levels of *candida* adherence and biofilm formation have been observed with heat-polymerized and milled manufactured discs [10]. Fiore et al. discovered similar comparable results: when comparing milled PMMA samples with heat-polymerized and additively constructed samples and concluded that the milled samples exhibited the lowest surface roughness prior to polishing and the lowest microbial adherence during the 90-minute incubation period [26].

In the context of additive manufacturing, the build angle, layer thickness, support structure orientation, printer type, and underlying printing technique all affect the quality and surface roughness of 3D-printed parts. The degree of polymerization, physical characteristics, surface roughness, and microbiological characteristics of the printed material are all influenced by all of the above factors [27, 39]. Other authors suggested the increased surface irregularities resulting from surface roughening contribute to the increase in fungal adhesion on roughened surfaces [25]. Whereas the study done by Wuersching, et al. indicates that in terms of surface roughness and microbial adherence, the 3D-printed occlusal guards performed overall suitable results. Thermoplastic materials appear to have rougher surfaces, increasing their vulnerability to microbial adherence. Additionally, for patients wearing dental appliances, it is crucial to comprehend how surface characteristics and microbial adherence interact in order to prevent caries and gingivitis. Moreover, following polishing, occlusal guard materials with thermoplastic qualities appear to have more microgrooves and a rougher surface. In conclusion, with regards to surface characteristics and bacterial biofilm formation, printed occlusal guard materials seem to be a good substitute for conventional powder/liquid-based resins and PMMA blocks [40]. Conventional polymerization of polymer materials is expected to have significant effects on the activity of microbial biofilm and affect the quantity of residual monomer in the material after processing. This feature has been demonstrated in experiments with *S. mutans* [41], and *C. albicans* [10].

Previous studies on prosthesis base resins found adhesion of *C. albicans* differed significantly between resins and manufacturing techniques. The surfaces of specimens produced additively had the maximum amount of *candida* adherence, while those manufactured subtractive had the lowest. whereas the samples that had been heat- and cold-cured acquired an intermediate position between the other two groups [42]. Additionally, Al-Fouzan et al. findings were made without the use of polishing techniques and discovered that *candida* adhesion to milled CAD/CAM and conventional heat-polymerized resins was positively correlated with surface roughness; *candida* adhesion to conventional resins was statistically higher than that of CAD/CAM polymers [9].

CAD/CAM dental polymers may be better suited for reducing microbial adherence (*C. albicans* and *S. aureus*) than conventional dental polymers, which were mostly affected by surface roughness independent of hydrophobicity during the fabrication of interim restorations and prostheses. Thermocycling may result in materials with rougher surfaces, less hydrophobicity, and altered microbial adherence [43].

In vivo applications affect the way microbial adherent to hard surfaces by the development of a pellicle derived from saliva substrates [44]. It is recognized that saliva and the interactions of different microbial biofilms create a very complex environment in the oral cavity, which may be important when analyzing the fungal adhesion to the various occlusal guard materials. To further comprehension of the microbiological behavior of these newly presented materials of an occlusal guard, clinical in vivo researches are highly recommended as a complement to the results of this in vivo study. Additional research examining the clinical behavior, process technology, and mechanical qualities of current study chrome cobalt alloy innovative material of occlusal guard constructed by investment casting technique is required to evaluate their applicability in the oral cavity.

5. Conclusion

While there were observed variations in *Candida* proliferation among the different occlusal guard materials tested, this study did not find a significant difference between the materials and *Candida* colonization in the oral cavity. However, the comparative analysis of the outcomes obtained after this study's first- and third-month periods of operation revealed the subjects with metal occlusal guard material were less prone to *Candida* colonization and exhibited the highest levels for conventional PMMA occlusal guard material. Further research with longer follow-up periods and larger sample sizes is required to better comprehend the impact of occlusal guard materials on *Candida* growth and to establish evidence-based guidelines for material selection in clinical practice.

This investigation was limited to individuals with different occlusal guards. These findings can aid immunocompromised patients in preventing the start of disease as much as possible. Such analysis could also be suitable for quantitative analysis of *Candida* concentration and monitoring the time-dependent changes.

Conflict of Interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

Not applicable.

Informed Consent

After receiving explanations about research and expressing their consent through signed terms of agreement and informed consent, the volunteers were allowed to take free participation in the study.

Availability of data and material

All data are available and upon request it will be sent to approved authors.

Authors' contributions

All authors contributed equally in this research study.

Funding
Non.

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