AVALIAÇÃO IN VITRO DA ADESÃO DE CANDIDA ALBICANS E CANDIDA PARAPSILOSIS ADHESION A MATERIAIS DE BASE DE PRÓTESES TIPO PROTOCOLO

In vitro evaluation of Candida albicans and Candida parapsilosis adhesion in protocol-type prosthesis bases

César Spielmann[1],
Andressa Gälzer[2],
Fernando Freitas-Portella[2],
Alexandra Flavia-Gazzoni[3],
Elken Gomes-Rivaldo[1]
https://zenodo.org/badge/DOI/10.5281/zenodo.10684904.svg

RESUMO

O gênero Candida tem sido mencionado como um dos principais causadores de infecções na cavidade bucal. Além da Candida albicans, outra espécie que tem sido identificada nas superfícies orais e sobre próteses dentárias é a Candida parapsilosis. O objetivo deste estudo foi avaliar a adesão de Candida albicans e Candida parapsilosis em materiais utilizados em bases de próteses tipo protocolo. As amostras foram divididas em 4 grupos. Grupo controle com amostras confeccionadas em resina acrílica termopolimerizável. Os demais grupos são compostos por amostras confeccionadas pela técnica CAD/CAM a partir de blocos de Cromo-cobalto, Titânio e Zircônia. As leveduras foram semeadas numa suspensão

[1] [1]Graduate Program in Dentistry, Universidade Luterana do Brasil. Av. Farroupilha, 8001, Canoas, RS, Brazil.
[2] Universidade Feevale. Rodovia RS-239 2755, Novo Hamburgo, RS, Brazil.
[3] Centro Universitário da Serra Gaúcha, Caxias do Sul, RS, Brazil.

fúngica a uma concentração de inóculo na 106 células/ml e incubadas a 37°C por 48 horas. Após a contagem, as unidades formadoras de colônias por ml foram fotografadas. Além disso, as amostras foram analisadas no microscópio eletrônico de varredura. Foi realizada análise descritiva referente as amostras de cada grupo e teste de comparação por meio da análise de variância com comparações múltiplas de Tukey, considerando-se significativo p<0,05. Também foram comparadas as espécies através da análise de variância. Os resultados mostraram que a Candida albicans aderiu significativamente menos no titânio do que nos outros três materiais. As Candida ssp tiveram diferentes comportamentos de colonização nos quatro materiais e verificada diferença significativa entre as espécies no titânio, acrílico e zircônia (p<0,05). Pode-se concluir que o titânio apresentou a menor adesão.

Palavras-chave: Candida albicans; Candida parapsilosis; prótese implanto-suportada; CAD/CAM; adesão.

ABSTRACT

The genus *Candida* has been mentioned as one of the main causes of infections in the oral cavity. In addition to *Candida albicans*, another species that has been identified on oral surfaces and dental prostheses is *Candida parapsilosis*. The aim of the present study was to evaluate the adhering of *Candida albicans* and *Candida parapsilosis* on different materials used in protocol type prosthesis bases. Samples were divided into three groups made by the CAD/CAM technique from cobalt-chromium, titanium and zirconium blocks added to an acrylic resin control group. The yeasts were seeded in a fungal suspension at an inoculum concentration of 10^6 cells/ml

and incubated at 37°C for 48 hours. After counting, the colony forming units per ml were photographed. In addition, the samples were analyzed under a scanning electron microscope. Descriptive analysis was performed for the samples in each group and a comparison test was performed using the analysis of variance with Tukey's multiple comparisons, considering $p<0.05$ as significant. *Candida albicans* adhered significantly less on titanium than on the other three materials. *Candida* ssp had a different colonization behavior in the four materials and a significant difference between the species was found in the titanium, acrylic and zirconium ($p<0.05$). It can be concluded that titanium presented the lowest adhesion.

Keywords: *Candida albicans*; *Candida parapsilosis*; implant-supported dentures; CAD/CAM, adhesion.

INTRODUCTION

Candida albicans is the most common yeast in the colonization of the oral cavity (SAMARANAYAKE; MACFARLANE, 1980, p. 611-615). while *Candida parapsilosis* is characterized as an important pathogen of nosocomial oral infections (PANAGODA; ELLEPOLA; SAMARANAYAKE, 2001, p. 29-35). Individual *Candida* species have different levels of biofilm formation, different morphologies and virulence, different production of lytic enzymes and different antifungal resistance (CAVALHEITO; TEIXEIRA, 2018, p. 1-15). Since the etiology of *Candida*-associated stomatitis is multifactorial, with several influencing parameters, a better understanding of the fundamentals of fungal adhesion is needed, this being obtained through the use of *in vitro* methods to study these adhesion processes. Microorganisms that adhere to dental materials can colonize other oral surfaces and

eventually cause oral infections in predisposed individuals (GÖKMENOGLU et al., 2018, p. 33-37).

The greater adhesion of *Candida parapsilosis* in relation to *Candida albicans* is justified because it has, as its main characteristic, a greater capacity for biofilm formation (CAVALHEITO; TEIXEIRA, 2018, p. 1-15). This is constituted by a cluster of yeast cells adhered to the surface with a minimal extracellular matrix (PEIXOTO et al., 2014, p. 75-82; SEABRA et al., 2013, p. 33-40). The surface roughness of the materials is the key factor for the deposition of the acquired film and the development of the plate (TEUGHELS et al., 2006, p. 68-81). The greater the surface roughness, the greater the plaque adhesion, and the increase in roughness increases the surface area for this fixation (AUSCHILL et al., 2002, p. 48–53). The use of scanning electron microscopy revealed great differences between the formation and growth of oral biofilm in different materials. In addition, microbiological analyses also showed significantly different results in the adhesion of these rougher surfaces (ENGEL et al., 2020, p. 162). The uneven topography and rough surfaces form a combination that provides a favorable interface for biofilm adhesion (HAO et al., 2018, p. 3157).

The surfaces of acrylic resins in complete denture bases manufactured with the CAD/CAM procedure showed promising potential to reduce the adhesion of *Candida albicans* compared to thermopolymerizable acrylic resins (AL-FOUZAN et al., 2017, p. 402-8). Furthermore, the development of digital systems allows the fabrication of fixed total dentures on implants through CAD/CAM procedures (PATZELT et al., 2013, p. 914-920). In this context, a protocol-type prosthesis base can be milled in a single piece (KAWAHATA et al., 1997, p. 540–548) in chromium, titanium and zirconia alloys (BILGIN et al., 2015, p. 576–579).

In addition to the CAD/CAM technology offering the possibility of obtaining a denture infrastructure with different materials, one of the great advantages of using these systems is the possibility of working with very resistant materials such as zirconia. The CAD/CAM technology was introduced in dentistry as a promising bet, enabling the manufacture of prostheses without compromising mechanical resistance (MIYAZAKI et al., 2009, p. 1-13).

The aim of the present study was to investigate *in vitro* the adhesion of *Candida albicans* and *Candida parapsilosis* on the surface of protocol-type prostheses bases made by CAD/CAM technology in titanium, cobalt-chromium and zirconia and to compare with that made in thermopolymerizable acrylic resin by the traditional technique.

MATERIALS AND METHODS

Forty-eight cylindrical specimens measuring 10mm in diameter x 3mm in thickness were made for the present study. Of these, 40 were divided into 4 groups, with n=10 (11) (Table 1) and, of the remaining 8, 2 samples from each group were separated for analysis under a scanning electron microscope.

Table 1. Experimental groups

Groups	Material	Composition
Acrylic resin (control)	**Conventional thermopolymerizable acrylic resin** (Vipi, Pirassununga, SP, Brazil)	**Powder:** Polymethylmethacrylate, Benzoyl Peroxide, Pigments. **Liquid:** Methylmethacrylate Monomer, Inhibitor, EDMA
Chrome cobalt	**Milled cobalt** (Sandinox Biometais, Sorocaba, SP, Brazil)	60% Cobalt, 1.5% Silicon, <1% Niobium, 28% Chromium, <1% Magnesium, 9% Tungsten.
Titanium	**Milled Titanium** (Realum, São Paulo, SP, Brazil)	0.05% Carbon, 0.08% Hydrogen, 0.015% Iron, 0.20% Aluminum, 5.5% - 6.75% Vanadium, Titanium 92.905% - 94.155% Titanium.
Zirconia	**Milled zirconia** (ProtMat, Juiz de Fora, MG, Brazil)	Óxido de zircônio 94-95%, Óxido de háfnio 2,1-5,0%, Óxido de alumínio ≤0,5%, Óxido de ítrio 4,5% - 6% 94-95% Zirconium Oxide, 2.1-5.0% Hafnium Oxide, ≤0.5% Aluminum Oxide, 4.5% - 6% Yttrium Oxide.

The samples from the control group were produced in thermopolymerizable acrylic resin from wax cylinders measuring 10mm x 3mm. The inclusion, pressing and finishing process was carried out following the manufacturer's recommendations. The other samples form the material groups of protocol-type prosthesis bases made by the CAD/CAM technique. Using the Exocad Dental software, a .nc file containing the sample design was generated. From this file and with the aid of a milling machine (Tecnodrill DM5 Novo Hamburgo RS, Brazil), it was possible to prepare the milled

samples. Group 2 had the samples made from a cobalt-chromium disc measuring 98.3mm in diameter x 10mm in thickness. The samples in Group 3 were made from a titanium disc measuring 98mm in diameter by 10mm in thickness. The average time for milling each sample in cobalt chromium was 29 minutes. The titanium samples had a milling time of around 18 minutes. They were polished according to the manufacturer's recommendations. In Group 4, the samples were made from a zirconia disc, measuring 98mm in diameter x 14mm in thickness, pressed and pre-sintered at 530°C to promote particle stabilization. Once the zirconia samples were made, with an average time of 13 minutes of milling for each one, the 10 samples went through the sintering process, now at a temperature range between 1400°C and 1580°C for 2 hours, with the final temperature of 1530°C. After this sintering, a polishing system with a high load of natural diamond (EVE Diapol) was used, with pre-polishing with the pink disk and the final polishing with the gray disk. All samples were measured with a Mitutoyo digital caliper (Japan).

The yeasts, both *Candida albicans* and *Candida parapsilosis* were seeded in 4% Sabourand Dextrose Agar and incubated at 37°C for 48 hours in a pure and new culture in a mycological greenhouse (DeLeo, model B 5 CBE Porto Alegre RS). In 48h, a new culture was obtained, where a fungal suspension was prepared at an inoculum concentration of 106 cel/ml, which corresponds to the 0.5 MacFarland scale. A Newbauer Camera was used for confirmation of concentration in an optical microscope (Olympus, model CX31 USA) at 40x magnification. The samples from all groups were washed with 70% alcohol and immersed separately in test tubes with a solution in the volume of 2 ml. Of these, 0.2 ml of suspension containing the inoculum at 106 cel/ml + 1.7 ml of 4% Sabouraud Dextrose Broth and 0.1 ml of artificial saliva, totaling 2 ml to cover the entire sample in the test tube. Incubation was carried out at 37°C in aerobiosis for 48 hours. Subsequently, the samples were

washed with 2 ml of sterile phosphate buffer saline to remove the planktonic yeasts that were around and would not form biofilm, and soon after, the specimens were transferred to sterile test tubes containing 2 ml of solution sterile physiological (NaCl 0.9%), where they were subjected to mechanical agitation with the aid of a Vortex shaker (Kasvi, Padova, Italy) for 1 minute to remove the fungal cells adhered to the specimens for counting only the yeasts, separating the planktonic ones. The suspensions obtained were then diluted and 100 µl of the suspension were seeded in Petri dishes containing Sabouraud Agar, and the cultures were incubated for 48 hours at a temperature of 37°C. Subsequently, the Colony Forming Units per milliliter (CFU/ml) were established and the counting was performed manually with the aid of a magnifying glass with 5x magnification with a flexible rod (model SP-CC-30, brand SPLABOR, Presidente Prudente-SP), and the photographic record of the images was also carried out with a Fuji camera, model FinePix S 5200 (China) to validate the results of the individual count of the formation of colonies on each plate.

The 8 samples previously separated were prepared, analyzed at magnifications of 400x, 4000x and 20000x and recorded on the EVO LS 10 Zeiss scanning electron microscope (Germany). Descriptive analysis was performed for the samples of each group and a comparison test between each material was performed using the Tukey's analysis of variance with multiple comparisons, considering $p<0.05$ as significant. *Candida* species were also compared using analysis of variance. All analyses were performed in the SPSS version 25 and in the R Program.

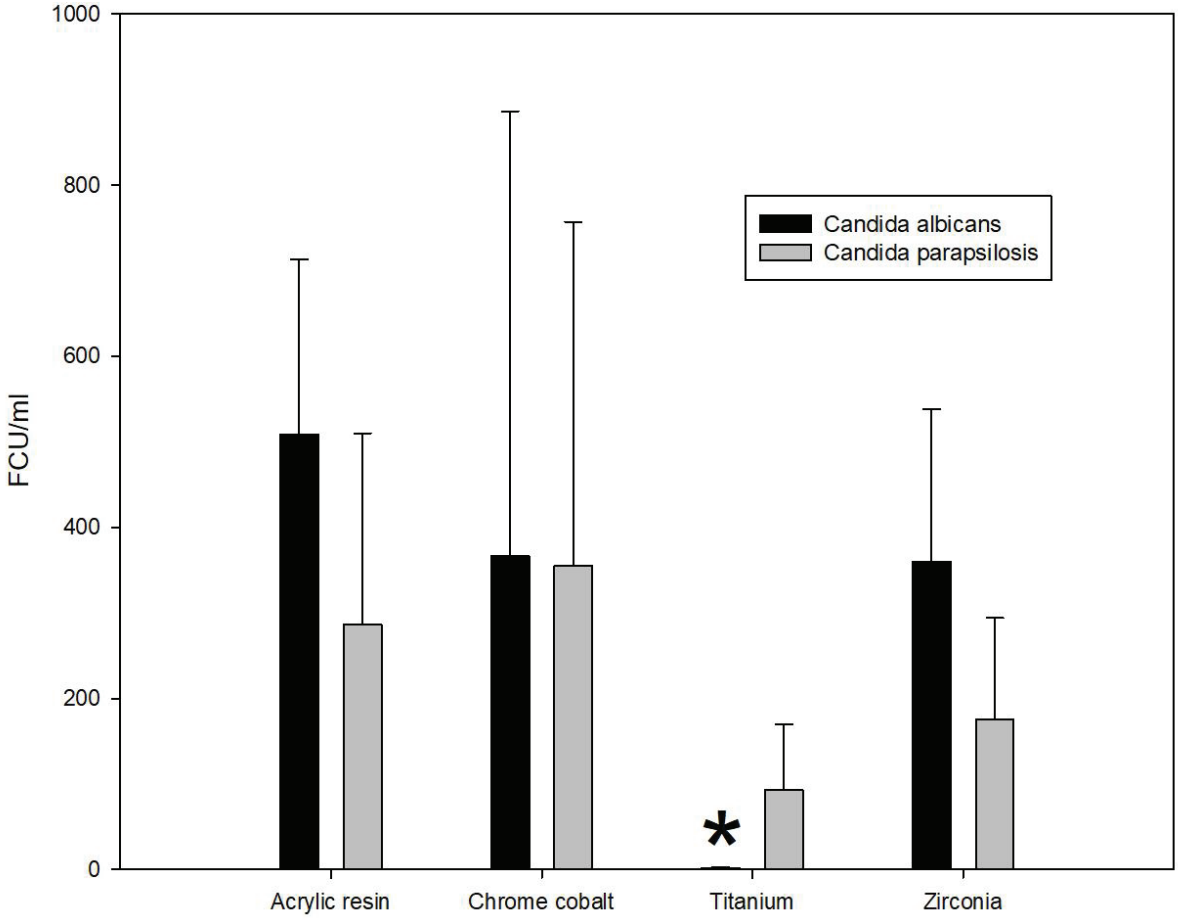
RESULTS

The pattern of the number of colony-forming units per ml in each species is described in Table 2. The titanium samples had a lower average number of colonies (mean=2.0 and sd=1.054), with a statistically significant difference ($p<0.05$) in relation to the other three materials for *Candida albicans* (Figure 1). When analyzing *Candida parapsilosis*, it was observed that the lowest values for adhesion were in the titanium (mean=92.60 and sd=77.556) and the highest values for the cobalt-chromium (mean=355.50 and sd=401.982), not finding a significant difference between all materials.

Table 2. Colony forming units (CFU/ml) as a function of *Candida albicans* and Candida *parapsilosis* species and disc materials.

		N	Mean	Standard deviation	95% confidence interval for mean		Minimum	Maximum
					Inferior limit	Superior limit		
Acrylic resin	*Candida albicans*	10	508.9	204.347	362.72	655.08	284	861
	Candida parapsilosis	10	286.4	223.76	126.33	446.47	18	658
Chrome cobalt	*Candida albicans*	10	366.2	52.023	328.98	403.42	271	433
	Candida parapsilosis	10	355.5	401.982	67.94	643.06	9	1038
Titanium	*Candida albicans*	10	2	1.054	1.25	2.75	1	4
	Candida parapsilosis	10	92.6	77.556	37.12	148.8	23	225
Zirconia	*Candida albicans*	10	360.2	178.1	232.8	487.6	156	730
	Candida parapsilosis	10	175.8	118.265	91.2	260.4	33	319

Figure 1 Number of colony-forming units (FCU) per ml of Candida albicans and Candida parapsilosis in the four materials: thermopolymerizable acrylic resin, cobalt chromium, titanium and zirconia. * indicates lower adhesion.



DISCUSSION

The colbalt chromium showed lower fungal adhesion compared to the other materials. The literature describes that *Candida* ssp adhesion basically depends on its ability to form biofilm (SARDI et al., 2013, p. 10-24). This adhesion to a solid surface is related to the interactions between the microbial cell and the surface (GU et al., 2015, p. 2348-9782). The roughness of intraoral hard surfaces can influence biofilm formation, where the

increase in surface roughness resulted in a simultaneous increase in biofilm formation (BOLLEN et al., 1997, p. 258-69).

The results showed that the acrylic resin samples showed the highest amount of colony formation per ml in *Candida albicans*. Confirming the results found, in other studies the thermopolymerizable acrylic resin presented a worse behavior than metals and ceramics (EGUIA et al., 2020, p. e13-e20). In its methodology, the biofilm formation was evaluated in 24 and 48 hours with the evaluation only of the *Candida albicans* adhesion (EGUIA et al., 2020, p. e13-e20; LI et al., 2013, p. 546-51). Low cost, ease of handling and aesthetics are among the main reasons for using thermopolymerable acrylic resin in protocol-type prosthetic bases (SINGH et al., 2013, p. 147–151). However, its main disadvantage is its roughness, which facilitates the adhesion of *Candida albicans* (BOLLEN et al., 1997, p. 258-69; CHATZIVASILEIOU et al., 2019, p. 196–197).

Eguia's work demonstrated similar behavior to that found in the present study, where titanium showed lower adhesion of *Candida albicans* compared to the cobalt-chromium and zirconia groups, even though the methodology was different in relation to the surface finish. Thermopolymerizable acrylic resins had 20x greater fungal adhesion on their surfaces (EGUIA et al., 2020, p. e13-e20).

Another study compared the adhesion of *Candida albicans* to titanium surfaces. Each group received different polishes and the group with the lowest surface roughness showed lower adhesion (MOUHAT et al., 2020, p. 146–157). Similar results were found in the study by Li, who evaluated the adhesion of *Candida albicans* according to surface polishing and observed under a scanning electron microscope (LI et al., 2013, p. 546-51). The amount of adhesion on the more polished surfaces was significantly lower than on other rougher samples. Surface topography images showed deeper

depressions, which could explain the greater biofilm formation (IONESCU et al., 2012, p. 458-465). The uneven topography and rough surfaces form a combination that provides a favorable interface for biofilm adhesion (WANG et al., 2014, p. e1–e16).

The greater corrosion of cobalt-chromium alloys when compared to titanium alloys provided an increase in roughness and, consequently, a greater biofilm formation (MYSTKOWSKA, 2016, p. 87–96; ZHANG et al., 2016, p. 78). The CAD/CAM technology used in the present study reduced the possibility of surface corrosion since the sample comes from milling an industrially manufactured disc. A lower adhesion capacity of *Candida albicans* to cobalt-chromium alloys compared to titanium was found (EGUIA et al., 2020, p. e13-e20), although it was not significant. Such difference can be explained taking into account the different compositions of materials and the finishing and polishing of surfaces (LI et al., 2013, p. 546-51; MOUHAT et al., 2020, p. 146–157).

In the study that investigated the formation and growth of oral biofilm *in vivo* (ENGEL et al., 2020, p. 162), the use of scanning electron microscopy revealed large differences between the formation and growth of biofilm in the studied materials compared to human enamel. Microbiological analyses also showed that significant differences were observed in the roughness of these materials. This demonstrates greater adhesion in cobalt-chromium metal alloys compared to composites, ceramics and tooth enamel as a control group. Although it was not the objective of the present study to evaluate the surface roughness, the scanning electron microscopy images showed that there is a relationship between the surface texture and the adhesion capacity of *Candida* ssp.

Since the present study has the limitation of being *in vitro*, other studies evaluating the count of colony-forming units per ml in Petri dishes could be

proposed, in addition to performing a count on all samples by SEM images, evaluating the surface roughness. Thus, this relationship between roughness and colony formation would be better clarified.

CONCLUSION

In evaluating the adhesion of *Candida albicans* and *Candida parapsilosis* on the surface of protocol-type prosthesis base materials, titanium was the material that presented the lowest adhesion.

Declaration of Conflicting Interests

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

Funding

None.

Correspondence

Fernando Portella. ERS-239, 2755. Novo Hamburgo, RS, Brasil. CEP 93525-075. E-mail: portellaff@yahoo.com.br.

REFERENCES

Al-Fouzan AF, Lamya AA, Ahmed M. Adherence of Candida to complete denture surfaces in vitro: A comparison of conventional and CAD/CAM complete dentures. J Adv Prosthodont, 2017; 9:402-8.

Auschill TM, Arweiler NB, Brecx M, Reich E, *et al*. The effect of dental restorative materials on dental biofilm. Eur J Oral Sci., 2002; 110(1):48–53.

Bilgin MS, Erdem A, Aglarci OS, Dilber E. Fabricating complete dentures with CAD/CAM and RP technologies. *J Prosthodont,* 2015; 24:576– 579.

Bollen CM, Lambrechts P, Quirynen M. Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: a review of the literature. Dent Mater,1997; 13(4):258-69.

Cavalheito M, Teixeira MC. *Candida* Biofilms: threats, challenges, and promising strategies. Frontiers in Medicine, 2018; 5(28):1-15.

Chatzivasileiou K, Kotsiomiti E, Vyzantiadis T-A. Effectiveness of Denture Cleansers on Removal of Adherent *Candida albicans* Cells from Denture Base Acrylics of Various Roughness. Int J Prosthodont, 2019; 32:196–197.

Eguia A, Arakistain A, De-la-Pinta I, López-Vicente J, *et al*. Candida Albicans Biofilms on Different Materials for Manufacturing Implant Abutments and Prostheses. Med Oral Patol Oral Cir Bucal., 2020; 25(1):e13-e20.

Engel AS, Kranz HT, Schneider M, Tietze JP, *et al*. Biofilm formation on different dental restorative materials in the oral cavity. BMC Oral Health., 2020; 20(1):162.

Gökmenoglu C, Kara NB, Beldüz M, Kamburoğlu A, *et al*. Evaluation of *Candida Albicans* biofilm formation on various parts of implant material surfaces. Nigerian Journal of Clinical Practice, 2018 21(1):33-37.
Gu W, Xu D, Sun S. *In vitro* models to study *Candida albicans* biofilms. Journal of Pharmaceutics & Drug Development, 2015; 3:2348-9782.

Hao Y, Huang X, Zhou X, Li M, *et al*. Influence of Dental Prosthesis and Restorative Materials Interface on Oral Biofilms. Int J Mol Sci., 2018; 19(10):3157.

Ionescu A, Wutscher E, Brambilla E. Influence of surface properties of resin-based composites on in vitro Streptococcus mutans biofilm development. Eur J Oral Sci., 2012; 120:458-465.

Kawahata N, Ono H, Nishi Y, Nagaoka E. Trial of duplication procedure for complete dentures by CAD/CAM. J Oral Rehabil., 1997; 24:540– 548.

Li Y, Guo T, Zhao J, Wang J. Effects of polishing methods on Candida albicans adhesion on cast pure titanium surfaces. Implant Dent., 2013; 22:546-51.

Miyazaki T, Hotta Y, Kunii J, Kuriyama S, *et al*. A review of dental CAD/CAM: current status and future perspectives from 20 years of experience. Dental materials journal, 2009; 1-13.

Mouhat M, Moorehead R, Murdoch C. *In vitro Candida albicans* biofilm formationon different titanium surfaces topographies. Biomater Investig Dent., 2020; 7(1):146–157.

Mystkowska J. Biocorrosion of dental alloys due to Desulfotomaculum nigrificans bacteria. *Acta Bioeng. Biomech.,* 2016; *18*:87–96.

Panagoda GJ, Ellepola ANB, Samaranayake LP. Adhesion of *Candida parapsilosis* to epitelial and acrylic surface correlateswith cell surface hydrophobicity. Mycoses, 2001; 44:29-35.

Patzelt SB, Vonau S, Stampf S, Att W. Assessing the feasibility and accuracy of digitizing edentulous jaws. J Am Dent Assoc., 2013; 144:914-920.

Peixoto JV, Rocha MG, Nascimento RTL, Moreira VV, *et al*. Candidíase - uma revisão de literatura. Brazilian Journal of Surgery and Clinical Research, 2014; 8(2):75-82.

Samarayake LP, MacFarlane TW. Factors affecting the *in vitro* adherence of *Candida albicans* to acrylic surfaces. Arch. Oral Biol., 1980; 25: 611-615.

Sardi JCO, Scorzoni L, Bernardi T, Fusco-Almeida AM, *et al*. Candida species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. J Med Microbiol., 2013; 62:10-24.

Seabra CL, Botelho CM, Henriques M, Oliveira R. Differential adherence and expression of virulence traits by *Candida albicans* and *Candida parapsilosis* in mono- and dual-species cultures in artificial saliva. Mycopathologia, 2013; 176(1-2):33-40.

Singh S, Palaskar JN, Mittal S. Comparative evaluation of surface porosities in conventional heat polymerized acrylic resin cured by water bath and microwave energy with microwavable acrylic resin cured by microwave energy. *Contemp Clin Dent.,* 2013; 4:147–151.

Teughels W, Van Assche N, Sliepen I, Quirynen M. Effect of material characteristics and/or surface topography on biofilm development. Clin Oral Implants Res., 2006; 17(2): 68-81.

Wang Z, Shen Y, Haapasalo M. Dental materials with antibiofilm properties. Dent. Mater., 2014; 30(2):e1–e16.

Zhang S, Qiu J, Ren Y. Reciprocal interaction between dental alloy biocorrosion and Streptococcus mutans virulent gene expression. *J. Mater. Sci. Mater. Med.,* 2016; *27*:78.