

Methods for *Candida albicans* biofilm formation on temporary soft liner

Métodos para a formação de biofilme de *Candida albicans* em reembasador macio temporário

DOI:10.34119/bjhrv4n5-234

Recebimento dos originais: 05/09/2021

Aceitação para publicação: 10/10/2021

Francine Sumie Morikava

Doutorado – Universidade Estadual de Ponta Grossa
Department of Dentistry, State University of Ponta Grossa – PR – Brazil
Av. General Carlos Cavalcanti, 4748, Uvaranas, Ponta Grossa – PR
E-mail: fransumie@gmail.com

Gustavo Simão Moraes

Mestrado – Universidade Estadual de Ponta Grossa
Department of Dentistry, State University of Ponta Grossa – PR – Brazil
Av. General Carlos Cavalcanti, 4748, Uvaranas, Ponta Grossa – PR
E-mail: moraes.gustavo29@yahoo.com.br

Victoria Schlumberger Cachoeira

Cirurgiã Dentista – Universidade Estadual de Ponta Grossa
Department of Dentistry, State University of Ponta Grossa – PR – Brazil
Av. General Carlos Cavalcanti, 4748, Uvaranas, Ponta Grossa – PR
E-mail: victoriacachoeira@hotmail.com

Mayara Precoma Ramalho

Cirurgiã Dentista – Universidade Estadual de Ponta Grossa
Department of Dentistry, State University of Ponta Grossa – PR – Brazil
Av. General Carlos Cavalcanti, 4748, Uvaranas, Ponta Grossa – PR
E-mail: mayaraprecoma@hotmail.com

Rafaela Alves da Silva

Doutorado – Faculdade de Odontologia de Bauru
Department of Prosthodontics and Periodontics, Bauru School of Dentistry, University of São Paulo – SP – Brazil
Al. Octávio Pinheiro Brisola 9-75, 17012-901, Bauru – SP
E-mail: rafaela@fob.usp.br

Karin Hermana Neppelenbroek

Doutorado – Faculdade de Odontologia de Bauru
Department of Prosthodontics and Periodontics, Bauru School of Dentistry, University of São Paulo – SP – Brazil
Al. Octávio Pinheiro Brisola 9-75, 17012-901, Bauru – SP
E-mail: khnepp@yahoo.com.br

Vanessa Migliorini Urban

Doutorado – Universidade Estadual de Ponta Grossa
Department of Dentistry, State University of Ponta Grossa – PR – Brazil
Av. General Carlos Cavalcanti, 4748, Uvaranas, Ponta Grossa – PR
E-mail: vanurban@yahoo.com

ABSTRACT

This study evaluated methods for the contamination of a soft liner material (Softone™) with *Candida albicans* biofilm. Specimens were either submitted or not to pretreatment in artificial saliva in an orbital incubator, and then held suspended in different positions (horizontal or vertical) and different storage conditions (bacteriological incubator or orbital incubator) during biofilm formation. Eight conditions were tested. All specimens were immersed in *C. albicans* inoculum and stored in an orbital incubator at 75 rpm or in a bacteriological incubator, both at 37°C for 90 min. Then, they were washed in PBS, and maintained in RPMI-1640 medium under the same conditions for 48 h. The degree of contamination was determined by the XTT assay. Data were submitted to ANOVA 1-factor/Tukey HSD test ($\alpha=0.05$). Specimens held horizontally in an orbital incubator showed the highest cell viability, while the ones kept vertically in a bacteriological incubator had the lowest viability ($p<0.0001$). The best condition for *C. albicans* biofilm formation was obtained when specimens were not submitted to pretreatment in saliva and were held horizontally in an orbital incubator.

Keywords: Artificial saliva, Denture liners, Denture stomatitis, Dental tissue conditioning.

RESUMO

Este estudo avaliou métodos para a contaminação de um material reembasador macio (Softone™) com biofilme de *Candida albicans*. As amostras foram submetidas ou não a pré-tratamento em saliva artificial em incubadora orbital, sendo então mantidas suspensas em diferentes posições (horizontal ou vertical) e em diferentes condições de armazenamento (incubadora bacteriológica ou incubadora orbital) durante a formação do biofilme. Oito condições foram testadas. Todos os espécimes foram imersos em inóculo de *C. albicans* e armazenados em incubadora orbital a 75 rpm ou em estufa bacteriológica, ambas a 37°C por 90 min. Em seguida, foram lavados em PBS e mantidos em meio RPMI-1640 nas mesmas condições por 48 h. O grau de contaminação foi determinado pelo ensaio XTT. Os dados foram submetidos ao teste ANOVA 1 fator / Tukey HSD ($\alpha = 0,05$). Os espécimes mantidos horizontalmente em incubadora orbital apresentaram a maior viabilidade celular, enquanto os mantidos verticalmente em estufa bacteriológica apresentaram a menor viabilidade ($p < 0,0001$). A melhor condição para a formação do biofilme de *C. albicans* foi obtida quando os espécimes não foram submetidos ao pré-tratamento na saliva e foram mantidos horizontalmente em incubadora orbital.

Palavras-chave: Saliva artificial, Reembasadores de Dentadura, Estomatite sob Prótese, Condicionamento de Tecido Mole Oral.

1 INTRODUCTION

Temporary soft liners have been widely used in dentistry as dynamic impression materials for patients who have irregular or extensively resorbed residual bone with a thin mucosal tissue, deep anatomic undercuts, bony protuberances, and those who cannot tolerate a hard denture base acrylic resin (Chladek et al. 2014, Krishnamurthy & Hallikerimath 2016). Given their ability to absorb some of the masticatory forces transmitted to the paraprosthodontic tissues (Verma et al. 2016), these materials are usually used to improve denture adaptation and retention (Ibraheem & ElGabry 2021), to treat and protect traumatized oral mucosa (Badaró et al. 2019), and to provide comfort after pre-prosthetic surgery or implant installation (Pereira-Cenci et al. 2008).

Soft liners can be classified according to their lifetime, as long or short-term materials, or according to their composition, as based on acrylic or silicone (ISO 1991). For temporary acrylic-based materials, the polymers are supplied as a powder containing polyethyl methacrylate or other copolymers (Rodrigues et al. 2013), which is mixed with a liquid containing 4 to 50 wt% ethyl alcohol and plasticizers (Rodrigues et al. 2013, Hong et al. 2020). When mixed, solvents diffuse between the polymer beads, which swell and then dissolve. The resulting material is formed of a gel with viscoelastic characteristics tangled polymer chains suitable for its clinical use (Hong et al. 2020). Since the powder contains no initiators and the liquid contains no monomers, these materials consist of non-crosslinked amorphous polymers. Due to pseudo entanglement of polymer chains, the material rapidly loses its viscoelastic properties with the release of alcohol and plasticizers into the oral environment (Rodrigues et al. 2013, Hong et al. 2020).

Consequently, temporary materials are easily degradable and susceptible to biofilm accumulation and *Candida* spp. Colonization (Pereira-Cenci et al. 2008), which may favor the development or worsening of pathological processes, such as denture stomatitis. In addition, the continuous swallowing or aspiration of microorganisms from the denture biofilm can expose patients, especially those immunocompromised or elderly, to systemic and life-threatening infections (Badaró et al. 2019, Ponde et al. 2021, de Paula et al., 2021).

Hence, the colonization and subsequent biofilm formation of *C. albicans* on soft lining materials is a major concern (Krishnamurthy & Hallikerimath 2016). Different studies (Hahnel et al. 2012, Krishnamurthy & Hallikerimath 2016, Vankadara et al. 2017, Baygar et al. 2018, Gulia et al. 2018, Badaró et al. 2019) have reported protocols for

biofilm formation on temporary soft liners, but there is no consensus regarding which method is the most appropriate. For instance, some studies have used saliva to enhance biofilm formation (Hahnel et al. 2012, Vankadara et al. 2017, Gulia et al. 2018), while others have not (Baygar et al. 2018, Badaró et al. 2019). In order to establish a reproducible in vitro contamination protocol, this study aimed to test different conditions for biofilm formation on soft liner specimens.

2 MATERIALS AND METHODS

2.1 MATERIAL AND EXPERIMENTAL GROUPS

Specimens (10x1 mm; n=6) were obtained with the temporary soft liner Softone™ (Bosworth Company, Skokie, IL, USA) for each experimental condition. The material was mixed according to the manufacturer's instructions, placed in sterile stainless-steel round molds and pressed between two sterile glass plates during setting time (Nikawa et al. 1993). The material excess was removed with a sterile spatula, and then the specimens were removed from the molds. The specimens were aseptically made in a laminar flow chamber (Pachane Indústria e Comércio Ltda., Piracicaba, SP, Brazil).

The specimens that were submitted to pretreatment in saliva (S) were placed in wells of 24-well culture plates containing 2 mL of artificial saliva (Table 1) (Hahnel et al. 2010) and were kept in an orbital incubator (OI; mod. 430/RDBP; Nova Ética, Vargem Grande Paulista, SP, Brazil) at 75 rpm and 37°C for 2 h (Hahnel et al. 2010). All saliva components were dissolved in phosphate-buffered saline solution (PBS; Sigma-Aldrich Inc., St. Louis, MO, USA) using a magnetic stirrer (model Q261-22; Quimis, Diadema, SP, Brazil), and the saliva was filtered with a 0.22 µm membrane using a sterile syringe.

Table 1. Artificial saliva composition

Component	Concentration	Manufacturer
Phosphate Buffered Saline (PBS)	Base (1000 mL)	Sigma-Aldrich
Bovine albumin	40 µg/mL	Sigma-Aldrich
α-amylase from hog pancreas	1 mg/mL	Fluka Bio-chemika
Lysozyme from hen egg	10 µg/mL	Fluka Bio-chemika
Pig Gastric mucin	850 mg/L	Sigma-Aldrich

The specimens were kept inside the culture plate wells, either vertically (V) or horizontally (H), attached by metallic devices (0.6 mm NiCr orthodontic wire; Morelli Ortodontia, Sorocaba, SP, Brazil) (Fig. 1) and stored in a bacteriological incubator (BI; MA 033; Marconi Equipamentos Laboratoriais Ltda., Piracicaba, SP, Brazil) or orbital incubator (OI; Shaker - mod. 430/RDBP; Nova Ética, Vargem Grande Paulista, SP, Brasil)

during biofilm formation. Eight experimental groups were then obtained: HOI, VOI, HBI, VBI, HOI-S, VOI-S, HBI-S, and VBI-S (Fig. 2).

Fig. 1 Metallic devices for maintaining the specimens vertically (A) or horizontally (B) inside the wells of the culture plate

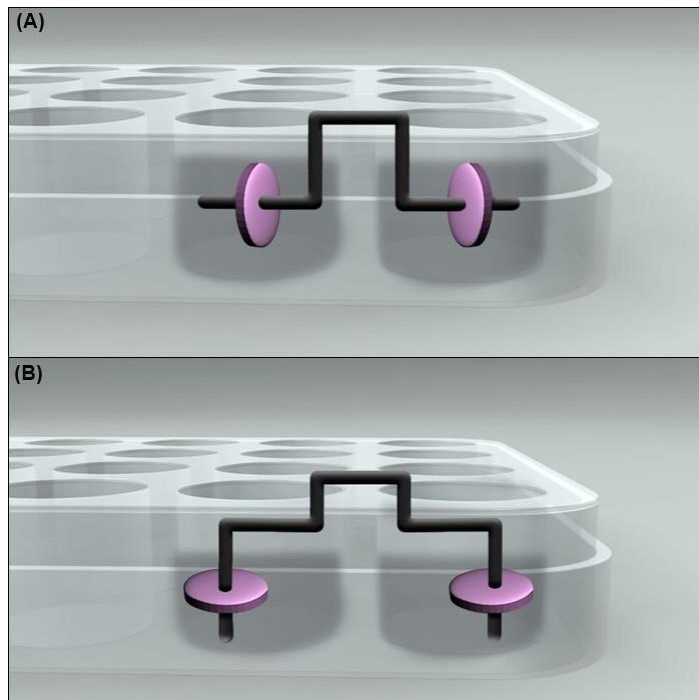
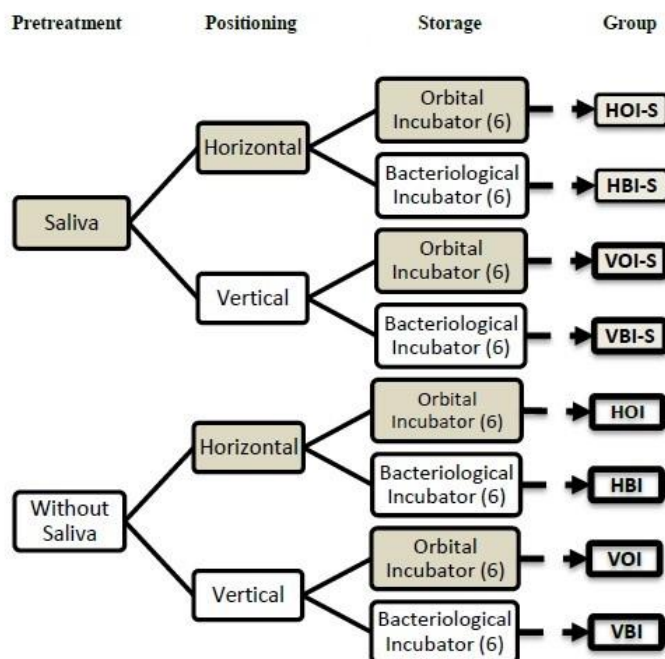


Fig. 2 Experimental conditions



2.2 PREPARATION OF THE INOCULUM

The wild strain of *C. albicans* SC5314 was stored at -80°C in a solution containing 20% glycerol (Kasvi Imp. e Dist., São José dos Pinhais, PR, Brazil) (Richards et al. 2008). One loop of the unfrozen strain was spread plated on a Petri dish (Difco®, São Paulo, SP, Brazil) containing YEPD agar (Clontech Laboratories Inc, Mountain View, CA, USA), which was incubated at 37°C for 48 h in a bacteriological incubator. Then, one colony of *C. albicans* was removed from the plate, resuspended in 20 mL of YEPD broth (Clontech Laboratories Inc) in Falcon tubes (TPP®; Techno Plastic Prod, Trasadingen, SH, Switzerland), and kept overnight (about 16 h) in a bacteriological incubator at 37°C, yielding a preculture.

For purification of cells, the Falcon tubes were centrifuged (mod. 5804 R; Eppendorf do Brazil Ltda., São Paulo, SP, Brazil) at 4,000 rpm for 15 min. The supernatant was discarded, and 5 mL of PBS was added to the pellet. The content was homogenized in vortex (mod. QL-901; Biomixer, Curitiba, PR, Brazil) followed by another centrifugation. This procedure was repeated once again (da Silva et al. 2008). Then, the final pellet was re-suspended in 20 mL of PBS.

The final pellet was vortexed and then serial dilutions were made at concentrations from 10^{-1} to 10^{-4} . Aliquots of 25 µL of these suspensions were spread plated on Sabouraud dextrose agar plates. This procedure was performed in duplicate. The plates were stored in a bacteriological incubator at 37°C for 48 h to confirm *C. albicans* colony forming units (CFU). The results were transformed to CFU/mL from the equation: $\text{CFU/mL} = \text{number of colonies} \times 10^n / q$; where “n” is the absolute dilution value (1, 2, 3, or 4), and “q” is the volume, in mL, pipetted for each dilution during plate seeding.

2.3 CELL ADHESION AND BIOFILM FORMATION

For cell adhesion, each specimen was individually placed in one well of a 24-well culture plate and immersed in 2 mL of inoculum ($\sim 2 \times 10^7$ CFU/mL). The culture plates were placed in an orbital incubator (HOI-S, VOI-S, HOI, and VOI groups) at a frequency of 75 rpm or in a bacteriological incubator (HBI-S, VBI-S, HBI, and VBI groups) both at 37°C for 90 min (Chandra et al. 2001). Then, the specimens were transferred to other wells containing 2 mL of PBS to remove non-adherent cells. Finally, they were immersed in 2 mL of RPMI-1640 (Sigma Aldrich) culture medium with 2% glucose and MOPS (Sigma Aldrich), where they remained during 48 h for biofilm formation (Kuhn et al. 2003).

2.4 TETRAZOLIUM SALT REDUCTION COLORIMETRIC ASSAY – XTT

The degree of contamination in each experimental group was determined according to the level of metabolic activity of viable cells in the biofilm in the XTT solution (Sigma Aldrich). The incubated specimens were removed from the RPMI-1640 culture medium and individually transferred to wells in a 24-well culture plate containing 2 mL of XTT solution prepared with menadione solution (da Silva et al. 2008). Then, all specimens were placed in an orbital incubator at a frequency of 75 rpm for 3 h at 37°C. After this period, an aliquot of 1.6 mL of the solution in each well was removed and transferred to microtubes (Kasvi, São José do Pinhais, PR, Brazil). These microtubes were then submitted to centrifugation at 10°C for 2 min, at a frequency of 10,000 rpm for cell decantation (Rotrosen et al. 1986, Kuhn et al. 2003). The supernatant of each microtube was transferred to a 96-well culture plate for evaluation of cell viability, which was measured in a spectrophotometer at 520 nm (Model Ultrospec 1000a; Amersham Pharmacia Biotech Inc.- Molecular Dynamics Div., Piscataway, NJ, USA).

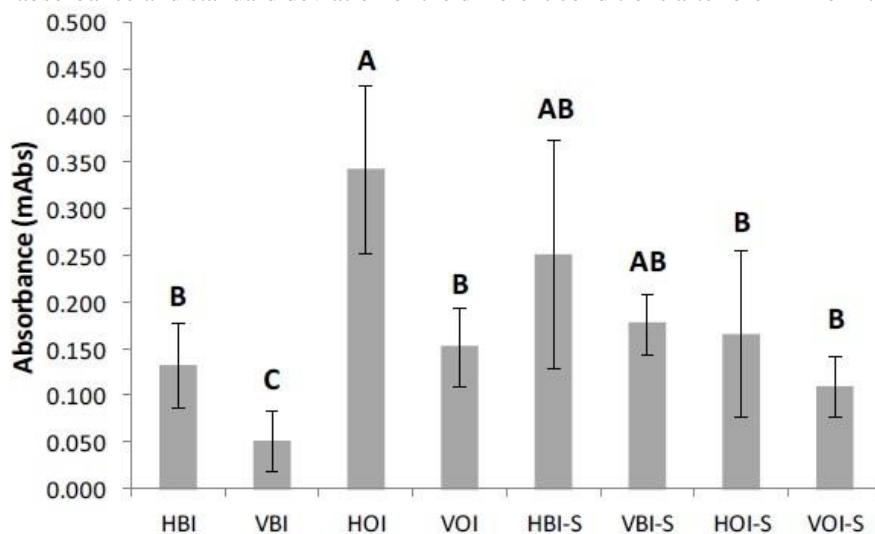
2.5 STATISTICAL ANALYSIS

Sample power analyzes were performed on data for the groups with and without pre-treatment in artificial saliva using G*Power 3.1.2 software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). The absorbance data (mAbs) were subjected to statistical analysis using one-way ANOVA followed by Tukey HSD test ($\alpha=0.05$).

3 RESULTS

For the number of specimens used ($n=6$), this study showed adequate power (100%, $\alpha=0.05$). The HOI group presented the highest mean absorbance and, consequently, the highest cell viability among the tested groups ($p<0.0001$). In contrast, the VBI group resulted in lowest cell viability ($p<0.0001$). HBI-S and VBI-S groups presented the intermediate absorbance values (Fig. 3).

Fig. 3 Mean absorbance and standard deviation of the different conditions after biofilm formation for 48 h



* Different upper-case letters indicate a statistically significant difference ($p < 0.05$)

4 DISCUSSION

The ability to form biofilms is an important pathogenic factor of *C. albicans* (Ponde et al. 2021). These biofilms are extremely resilient and hard to eradicate and can be described as an organized microbial community of highly heterogeneous and three-dimensional architecture consisting of host cells, yeast, hyphae, pseudohyphae, and an extracellular matrix (Tobudic et al. 2012, Nett & Andes 2020, Ponde et al. 2021). The development of *C. albicans* biofilm consists of four consecutive stages: 1) adherence of yeast cells to the substrate; 2) cell proliferation and production of the extracellular matrix, which provides protection from external threats; 3) biofilm maturation; and 4) dispersion of cells from the mature biofilm (Nett & Andes 2020, Mourer et al. 2021, Ponde et al. 2021).

Soft liners are reportedly more susceptible to candidal colonization than acrylic materials (Hahnel et al. 2012). Some factors that may contribute for their contamination include the difficult cleaning and disinfection (Baygar et al. 2018, Badaró et al. 2019), surface roughness (Sánchez-Aliaga et al. 2021), bond strength, surface texture/porosity, hardness, and chemical composition (Bulad et al. 2004, Pereira-Cenci et al. 2008). Some commercial soft liners have a relatively low antimicrobial activity, which can be attributed to their components (Krishnamurthy & Hallikerimath 2016). The Softone™ liquid contains butyl benzoate and dibutyl phthalate as plasticizers. In another study, dibutyl phthalate presented a lower contamination than benzyl n-butyl phthalate, butyl phthalyl butyl glycolate, and benzyl salicylate specimens (Nikawa et al. 1995). This might be the reason for the initial low contamination of the Softone™ specimens observed in the

present study, suggesting that the material composition might be related to its susceptibility to fungal contamination.

The role of saliva during the initial colonization and subsequent *C. albicans* biofilm formation is a complex and poorly understood subject, especially taking the surface of soft liner materials into account (Nikawa et al. 2000, Pereira-Cenci et al. 2008). Different types and compositions of both natural and artificial saliva have been tested aiming to improve biofilm formation on soft liners (Nikawa et al. 1993, Pereira-Cenci et al. 2008, Hahnel et al. 2012, Vankadara et al. 2017, Gulia et al. 2018). However, there is no consensus regarding which type of saliva is most appropriate for biofilm formation on these materials. Some authors have shown that specimen immersion in whole saliva obtained from a healthy individual has compromised the initial adherence of *C. albicans* (Pereira-Cenci et al. 2008), while others have reported otherwise (Hahnel et al. 2012, Gulia et al. 2018).

This is the first study to evaluate the effect of saliva immersion on the initial adhesion of *C. albicans* to the Softone™ material. The saliva pretreatment only resulted in greater adhesion of *C. albicans* for the VBI-S group in comparison to the VBI group. The artificial saliva used in the present study presents a similar composition to the human whole saliva and has been used in several studies to colonize microorganisms, including *C. albicans* (Hahnel et al. 2010). Although statherin and proline-rich proteins interact with *Candida* cell walls and increase their adsorption to polymeric surfaces, histatins, lactoferrins, and lysozyme (one of the components of the artificial saliva used in this study) inhibit *Candida* adherence by blocking access to important nutrients or by attaching to their adhesins (ten Cate et al. 2009).

The assessment of the effect of specimen positioning on biofilm formation is justified by the fact that gravity could positively influence the adhesion of *Candida* in the horizontally positioned specimens. There was also a significant difference between the HOI-S and HOI groups, with the latter showing the highest contamination values from all the specimens. During the initial fungal adhesion period, the specimens were either subjected to agitation in an orbital incubator or not. These conditions were tested considering that under conditions of dynamic growth, such as the use of an orbital or rotary shaker or another source of shear force, the development of biofilm on the specimens could be hampered (Pereira-Cenci et al. 2008).

The XTT method is a colorimetric assay used to evaluate cellular physiology in biofilms (Azeredo et al. 2017) that has the advantages of being rapid, highly reproducible,

non-invasive, and requiring minimal post-processing of samples when compared to other methods (Ramage 2016). Despite some limitations, including the variability in readings when comparing different species, this technique is useful for direct comparisons and is considered the primary tool when it comes to investigating candidal biofilms (Ramage 2016).

Further research is still needed to fully elucidate the mechanisms of biofilm formation in soft liners with polymicrobial communities, which are related to more challenging infections (Ponde et al. 2021). In addition, different types of saliva, both natural and artificial, could be compared. Regardless of the limitations related to in vitro experiments, the horizontal positioning of specimens in an orbital incubator without the saliva pretreatment was the best condition for biofilm formation.

ACKNOWLEDGEMENTS AND FUNDING

The authors would like to thank Christina Desnoyers (University of Saskatchewan, Canada) for her writing assistance and Henrique Moraes (State University of the Midwest of Paraná, Brazil) for his help with the illustrations. This work was supported by the Brazilian National Council for Scientific and Technological Development – CNPq (MCTI/CNPq/Universal edict 14/2014 A – Grant # 456403/2014-0) and the São Paulo Research Foundation – FAPESP (Grant # 2017/07314-1).

REFERENCES

- Azeredo J, Azevedo NF, Briandet R, Cerca N et al. 2017 – Critical review on biofilm methods. *Critical Reviews in Microbiology* 43, 313-351.
- Badaró MM, Leite-Fernandes VMF, Martin LT, Oliveira VC et al. 2019 – Antibiofilm Activity of an Experimental *Ricinus Communis* Dentifrice on Soft Denture Liners. *Brazilian Dental Journal* 30, 252-258.
- Baygar T, Ugur A, Sarac N, Balci U et al. 2018 – Functional denture soft liner with antimicrobial and antibiofilm properties. *Journal of Dental Sciences* 13, 213-219.
- Bulad K, Taylor RL, Verran J, Mccord JF. 2004 – Colonization and penetration of denture soft lining materials by *Candida albicans*. *Dental Materials* 20, 167-175.
- Chandra J, Mukherjee PK, Leidich SD, Faddoul FF et al. 2001 – Antifungal resistance of candidal biofilms formed on denture acrylic *in vitro*. *Journal of Dental Research* 80, 903-908.
- Chladek G, Żmudzki J, Kasperski J. 2014 – Long-Term Soft Denture Lining Materials. *Materials (Basel)* 7, 5816-5842.
- da Silva WJ, Seneviratne J, Parahitiyawa N, Rosa EA et al. 2008 – Improvement of XTT assay performance for studies involving *Candida albicans* biofilms. *Brazilian Dental Journal* 19, 364-369.
- de Paula CC, Passos JPC, Vieira FC, da Silva ENC et al. 2021 – Colonization of fungal genes in positive hemocultures of hospitalized patients. *Brazilian Journal of Health Review* 4, 9952-9963.
- Gulia S, Bhatt V, Shetty M, Prasad KD et al. 2018 – Effect of Type II Diabetes Mellitus, *Candida Albicans* and *Streptococcus Mutans* on the Biofilm Formation on Prosthetic Materials. *The Journal of Contemporary Dental Practice* 19, 1538-1545.
- Hahnel S, Ettl T, Gosau M, Rosentritt M et al. 2010 – Influence of saliva substitute films on the initial adhesion of *Candida albicans* to dental substrata prior to and after artificial ageing. *Archives of Oral Biology* 55, 391-396.
- Hahnel S, Rosentritt M, Bürgers R, Handel G et al. 2012 – *Candida albicans* biofilm formation on soft denture liners and efficacy of cleaning protocols. *Gerodontology* 29, e383-391.
- Hong G, Wang WQ, Sun L, Han JM et al. 2020 – The Dynamic Viscoelasticity of Dental Soft Polymer Material Containing Citrate Ester-Based Plasticizers. *Materials (Basel)* 13.

- Ibraheem EM, Elgabry HS. 2021 – Effect of mandibular complete dentures relining on occlusal force distribution using T-scan system. Bulletin of the National Research Centre 45, 93.
- ISO. 1991 – International Organization for Standardization: Specification 10139-1: resilient lining materials for removable dentures - part 1 - short-term materials, Switzerland, ISO.
- Krishnamurthy S, Hallikerimath RB. 2016 – An *In-vitro* Evaluation of Retention, Colonization and Penetration of Commonly Used Denture Lining Materials by *Candida albicans*. Journal of Clinical and Diagnostic Research 10, Zc84-zc88.
- Kuhn DM, Balkis M, Chandra J, Mukherjee PK et al. 2003 – Uses and limitations of the XTT assay in studies of *Candida* growth and metabolism. Journal of Clinical Microbiology 41, 506-508.
- Mourer T, El Ghalid M, D'enfert C, Bachellier-Bassi S. 2021 – Involvement of amyloid proteins in the formation of biofilms in the pathogenic yeast *Candida albicans*. Research in Microbiology 172, 103813.
- Nett JE, Andes DR. 2020 – Contributions of the Biofilm Matrix to *Candida* Pathogenesis. Journal of Fungi (Basel), 6.
- Nikawa H, Hayashi S, Nikawa Y, Hamada T et al. 1993 – Interactions between denture lining material, protein pellicles and *Candida albicans*. Archives of Oral Biology 38, 631-634.
- Nikawa H, Jin C, Hamada T, Makihiro S et al. 2000 – Interactions between thermal cycled resilient denture lining materials, salivary and serum pellicles and *Candida albicans in vitro*. Part II. Effects on fungal colonization. Journal of Oral Rehabilitation 27, 124-130.
- Nikawa H, Yamamoto T, Hamada T. 1995 – Effect of components of resilient denture-lining materials on the growth, acid production and colonization of *Candida albicans*. Journal of Oral Rehabilitation 22, 817-824.
- Pereira-Cenci T, Del Bel Cury AA, Crielaard W, Ten Cate JM. 2008a – Development of *Candida*-associated denture stomatitis: new insights. Journal of Applied Oral Science 16, 86-94.
- Pereira-Cenci T, Deng DM, Kraneveld EA, Manders EM et al. 2008b – The effect of *Streptococcus mutans* and *Candida glabrata* on *Candida albicans* biofilms formed on different surfaces. Archives of Oral Biology 53, 755-764.
- Ponde NO, Lortal L, Ramage G, Naglik JR et al. 2021 – *Candida albicans* biofilms and polymicrobial interactions. Critical Reviews in Microbiology 47, 91-111.

- Ramage G. 2016 – Comparing apples and oranges: considerations for quantifying candidal biofilms with XTT [2,3-bis(2-methoxy-4-nitro-5-sulfo-phenyl)-2H-tetrazolium-5-carboxanilide] and the need for standardized testing. *Journal of Medical Microbiology* 65, 259-260.
- Richards TS, Oliver BG, White TC. 2008 – Micafungin activity against *Candida albicans* with diverse azole resistance phenotypes. *The Journal of Antimicrobial Chemotherapy* 62, 349-355.
- Rodrigues S, Shenoy V, Shetty T. 2013 – Resilient liners: a review. *Journal of Indian Prosthodontic Society* 13, 155-164.
- Rotrosen D, Calderone RA, Edwards JE Jr. 1986 – Adherence of *Candida* species to host tissues and plastic surfaces. *Reviews of Infectious Diseases* 8, 73-85.
- Sánchez-Aliaga A, Farago PV, Michél MD, Sugio CYC et al. 2021 – Surface morphology and in vitro leachability of soft liners modified by the incorporation of antifungals for denture stomatitis treatment. *Journal of Applied Oral Science* 28, e20200639.
- ten Cate JM, Klis FM, Pereira-Cenci T, Crielaard W et al. 2009 – Molecular and cellular mechanisms that lead to *Candida* biofilm formation. *Journal of Dental Research* 88, 105-115.
- Tobudic S, Kratzer C, Lassnigg A, Presterl E. 2012 – Antifungal susceptibility of *Candida albicans* in biofilms. *Mycoses* 55, 199-204.
- Vankadara SK, Hallikerimath RB, Patil V, Bhat K et al. 2017 – Effect of *Melaleuca alternifolia* Mixed with Tissue Conditioners in Varying Doses on Colonization and Inhibition of *Candida albicans*: An *In Vitro* Study. *Contemporary Clinical Dentistry* 8, 446-450.
- Verma M, Menghani S, Devi J, Gupta R et al. 2016 – A Novel Approach to Treat Traumatized Alveolar Ridges: Two Case Reports. *Case Reports in Dentistry* 2016, 9312412.