Guanidine derivative inhibits C. albicans biofilm growth on denture liner without promote loss of materials' resistance

Isadora Martini Garcia[a], Stéfani Becker Rodrigues[a], Maria Eduarda Rodrigues Gama[a], Vicente Castelo Branco Leitune[a], Mary Anne Melo[b,c], Fabrício Mezzomo Collares[a,*]

[a] Dental Materials Laboratory, Postgraduate Program in Dentistry, School of Dentistry, Federal University of Rio Grande Do Sul, Rua Ramiro Barcelos, 2492, Rio Branco, 90035-003, Porto Alegre, RS, Brazil
[b] Ph.D. Program in Biomedical Sciences, University of Maryland School of Dentistry, Baltimore, MD, 21201, USA
[c] Operative Dentistry Division, General Dentistry Department University of Maryland School of Dentistry, Baltimore, MD, 21201, USA

A B S T R A C T

To reduce the burden of denture stomatitis and oral candidiasis, an aqueous solution containing poly-hexamethylene guanidine hydrochloride (PHMGH) was investigated as an antifungal disinfectant against the leading cause of these oral conditions, *Candida albicans*. The solutions formulated with concentrations ranging from 0.125 to 0.50 wt% enabled increasing disinfection at the initial 5min-contact with 72h-mature candida biofilms formed on denture liner specimens. After 10 min-contact, the solution at lower concentration has reached total fungal elimination. The results also indicated that the denture liners preserved their mechanical property after the maximum contact time with the solution at the highest tested concentration. The PHMGH aqueous solutions at 0.125 wt% could be applied to promote interim denture liner disinfection without promoting the loss of materials' mechanical property.

1. Introduction

Oral candidiasis is more prevalent in denture wearers, im-munocompromised, and patients under long-term use of antimicrobial drugs [1]. Fungal oral biofilms, especially those caused by candida species, are very difficult to eradicate due to their complex structure and recalcitrance [2,3]. Further, oral structures are prone to be colo-nized. Dental materials used for dentures and intended to intraoral use are often associated with a high rate of infections due to their porosity and susceptible surface morphology [2,3].

A significant proportion (72%) of complete denture wearers [4] is affected by denture stomatitis. It has been estimated that 93% [5,6] of these oral infections are linked to *Candida albicans* colonization and it is associated with deficient oral and denture hygiene, high roughness of denture liner surface, and trauma [7,8]. Furthermore, it is one of the most common microorganisms related to nosocomial pneumonia (NP) and ventilator-associated pneumonia (VAP), increasing the costs to health care annually [9].

Acrylics denture liners are widely used to improve the comfort of complete and partial removable denture wearers once they act as a cushion on the tissue surface [10]. Denture liners absorb the impact of masticatory forces and assist in the soft tissue tension distribution [11–14]. Despite their benefits, denture liner materials present inherent drawbacks, including rapid reduce of softness, permanent deformation, high porosity, low tear strength and high rates of candida-driven fungal infections [11,15–17]. The denture liners are easily contaminated in the oral environment due to inherent surface porosity. These intrinsic re-tentive areas of the material make the mechanical removal by brushing very challenge [18]. Denture disinfectant agents such as chlorhexidine gluconate, sodium hypochlorite and hydrogen peroxide have failed to reach complete disinfection without compromise the physical and chemical properties of denture liners [19,20].

Polyhexamethylene guanidine hydrochloride (PHMGH), an anti-microbial agent of the guanidine family, is an effective antimicrobial polymer against gram-positive and gram-negative bacteria, virus, fungi [21,22]. Its antibacterial effectiveness has been reported against clini-cally relevant and high antibiotic-resistant microorganisms such as those denominated ESKAPE pathogens (*Enterococcus faecium, Staphy-lococcus aureus, Klebsiella pneumoniae, Actinetobacter baumannii, Pseu-domonas aeruginosa* and *Enterobacter species*) [23]. PHMGH also has

shown high water solubility [23]. Besides, previous studies mentioned their lack of dyes and odor which makes this agent very suitable for dental application [23–25]. Based on that, researchers [26] have recently investigated the effect of PHMGH incorporated in a dental resin-based formulation. The incorporation of this compound led to lower viability of caries-linked bacteria while maintaining reliable physical and chemical properties of the dental resin.

Based on it, the present study investigates a rapid, simple, and effective approach for the inhibition of mature biofilm formation of *Candida albicans* over denture liners by immersion in solutions with different concentrations of guanidine derivative (PHMGH) and the consequent effects on their mechanical property.

2. Materials and methods

2.1. PHMGH solutions formulation

Polyhexamethylene guanidine hydrochloride (PHMGH) was obtained without further purification and used for the preparation of water-based solutions at concentrations below 1% ranging from 0.125 wt% to 0.5 wt%. For that, PHMGH solutions were vortexed and one flask with sterile distilled water remained without PHMGH addition for the control group. The following groups were formulated: Control group (G_{CTRL}), $PHMGH_{0.125\%}$, $PHMGH_{0.25\%}$ and $PHMGH_{0.5\%}$. The chemical structure of PHMGH is presented in Fig. 1.

2.2. Preparation of specimens for antifungal activity evaluation

The powder and the liquid of Coe-comfort (Coe Laboratories Inc., Chicago) were manually mixed according to the manufacturer's instructions. Denture liner specimens were prepared for the antifungal activity evaluation, under aseptic conditions and sterile instruments. A metallic mold with 64 mm long, 10 mm wide and 3 mm thick was used to create rectangular specimens. The specimens were then measured with a digital caliper (Mitutoyo, Kawasaki, Kanagawa, Japan). Square-shape specimens were created with 60 mm^2 each one after the cutting. The specimens were exposed to ultraviolet energy for 30 min on one side and more than 30 min on the opposite side in laminar airflow. Then, they were exposed to saliva (section 2.4) and *Candida albicans* (section 2.5) contact.

2.3. Microorganism and growth conditions

Candida albicans (ATCC 10231) was reactivated from the original culture with 10 mL of brain-heart infusion broth (BHI) (Aldrich Chemical Co.) with 0.5 wt% of glucose for 24 h at 37 °C. After the incubation period, 100 μL of medium with *C. albicans* were platted on Sabouraud Dextrose agar in Petri dishes and was kept at 37 °C for 24 h. A bacteriological loop was filled twice with the microorganism and immersed in 25 mL of BHI with 0.5 wt% of glucose for 24 h at 37 °C, which corresponded to 1.5×10^7 cells/mL. The incubation periods were performed under microaerophilic environment.

2.4. Formation of the acquired pellicle on acrylic surfaces

To simulate intraoral conditions and promote microorganism adhesion, an artificially acquired pellicle was created over the specimens. Under institutional research board approval by the local Ethics Committee (2.780.491), human saliva was collected. A healthy volunteer aged 25 years, who had not used antibiotics, mouth rinses or any other medication, participated in the study providing stimulated saliva. The collected saliva passed through 0.22 μm-filter (Millipore Corporation) with phosphate-buffered saline (PBS) solution (KCl at 50 mM, KPO_4 at 1 mM, $CaCl_2$ at 1 mM, $MgCl_2$ at 0,1 mM, pH 6,5) at 1:1. The specimens were kept under UV for 30 min on each side and placed in sterile centrifuge tubes with 1 mL of saliva for 30 min at 37 °C to form the acquired pellicle.

2.5. Adherence of C. albicans

After the formation of the acquired pellicle of saliva, the specimens were removed from the tubes, transferred to sterile centrifuge tubes with 1 mL of the previously prepared suspension of *C. albicans*, and incubated at 37 °C for 2 h (to yeast adherence phase). For biofilm maturation, the specimens were washed with saline solution (0.9% NaCl) and transferred to a new centrifuge tube containing BHI broth and 0.5 wt% of glucose for 72 h at 37 °C aerobically. Media were renewed every 24 h to form 72 h-mature biofilms on denture liner specimens.

2.6. Antifungal activity of PHMGH solutions in mature biofilms

The infected specimens were dipped at PHMGH solutions for 5 min
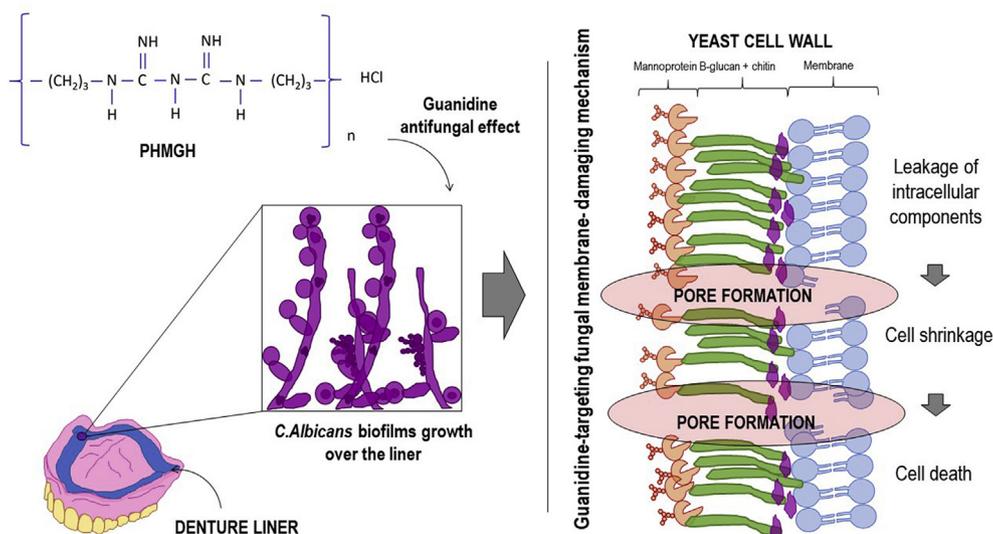

Fig. 1. Schematic drawing of the suggested mechanism of action of PHMGH solutions on Candida albicans biofilm. Upon contact, PHMGH triggers a membrane damage pathway leading to fungal cell shrinkage and death.

(n = 3) or 10 min (n = 3). G_{CTRL} remained without PHMGH solution contact. The specimens were washed with 5 mL of saline solution (0.9% NaCl), transferred to sterilized centrifuge tubes and vortexed during 1 min in 1 mL of saline solution (0.9% NaCl) with sterile glass pearls to allow the biofilm structure disruption. The vortexed solutions were diluted until the 10^{-6} dilution in saline solution (0.9% NaCl). Two drops (25 μL each) of each dilution were plated on Sabouraud Dextrose agar in Petri dishes and aerobically incubated for 36 h at 37 °C to visually count the colonies. The number of colony-forming units (CFUs) per milliliter was calculated according to the dilution and transformed to log CFU/mL. The dilution and the plating were performed using graduated pipettes.

2.7. Tensile strength evaluation

To test the mechanical property of the liners after their contact with the solutions (distilled water or distilled water with PHMGH), five samples per group were prepared (n = 5). First, the powder and the liquid of GC COE COMFORT® were mixed at 6:5 by weight, according to the manufacturer's instruction. Each mix of denture liner was placed into a silicone mold, according to ASTM D638-02a [27]. The mold was placed between two glass plates and the pressure was applied to extrude excess materials and to remove air bubbles. Then, a load of 1 kg was applied above the set.

Each sample was removed from the mold and placed in a distilled water bath at 37 °C for 40 h to complete the setting. Then, the samples were immersed in the different solutions containing PHMGH ($PHMGH_{0.125\%}$, $PHMGH_{0.25\%}$ and $PHMGH_{0.5\%}$) or pure distilled water (G_{CTRL}) for 5 or 10 min. After the contact with the solutions, the samples were placed in a metallic device to pull each one and test their tensile strength in a universal testing machine (EZ-SX Series, Shimadzu). The crosshead speed used was of 500 ($\pm$ 50) mm/min according to ASTM D638-02a [27]. The samples were tested up to their failure of tensile strength and the maximum value was calculated in megapascals (MPa).

2.8. Statistical analysis

Data distribution was evaluated using the Shapiro-Wilk test and the values were analyzed with two-way ANOVA and Tukey's post hoc test. A level of significance of 0.05 was considered for all tests performed.

3. Results

After 10 min of immersion of the specimens in any PHMGH solution, there were no detectable colonies formed on agar plates (Fig. 2). After this period, all aqueous solutions containing PHMGH promoted less than 10 colonies of *C. albicans* per agar plate. The CFU/mL was established as 1 for specimens that presented no detectable colonies on Sabouraud Dextrose agar to achieve at least the result "0" after logarithmic transformation, to be possible to represent the values in log. When compared 5–10 min of immersion, there was lower biofilm after 10 min for $PHMGH_{0.250\%}$ and $PHMGH_{0.125\%}$ ($p < 0.05$). As the higher the concentration of PHMGH at 5 min evaluation, the lower was the detectable colonies.

The tensile strength ranged from 0.19 ($\pm$ 0.02) MPa for the control group after 10 min to 0.24 ($\pm$ 0.05) MPa for $PHMGH_{0.250\%}$ after 5 min of immersion (Fig. 3). There was no statistically significant difference among groups for tensile strength after immersion in any PHMGH solutions in the different periods ($p > 0.05$).

4. Discussion

Here, we have investigated and observed the antifungal effect of an aqueous solution containing PHMGH for denture liners for the first time. After 10 min-contact, the solution at lower concentration has
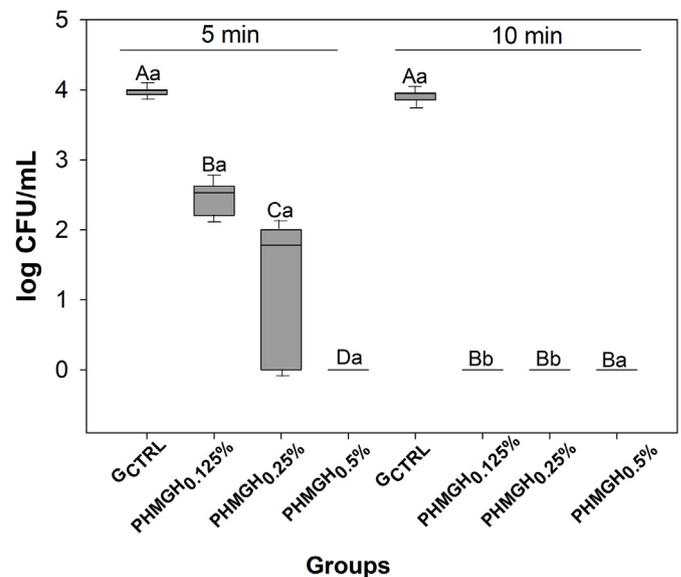

Fig. 2. Graph of mean and standard deviation values of log CFU/mL of antifungal activity evaluation of PHMGH solutions in mature biofilms of *Candida albicans* in denture liner samples. Different capital letters indicate statistical difference among groups in the same time of contact - 5 or 10 min ($p < 0.05$). Different lowercase letters indicate statistical difference for the same group at different times of contact – 5 or 10 min ($p < 0.05$).
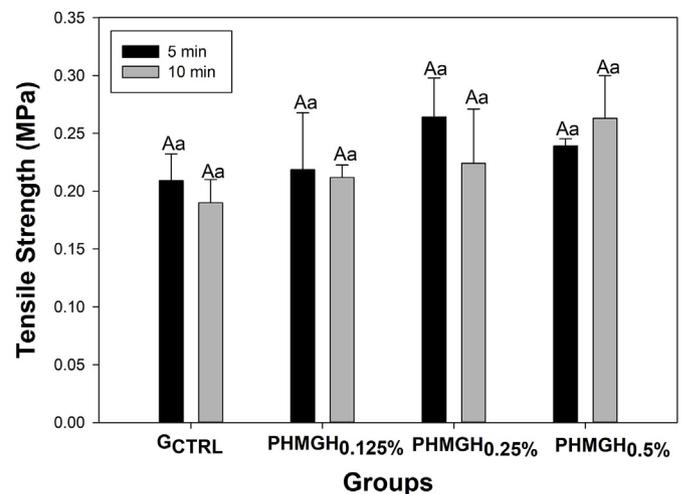

Fig. 3. Graph of mean and standard deviation values for tensile strength according to different concentrations of PHMGH and immersion times. Same capital letters indicate no statistical difference among groups in the same time of contact - 5 or 10 min ($p > 0.05$). Same lowercase letters indicate no statistical difference for the same group at different times of contact – 5 or 10 min ($p > 0.05$).

reached total fungal elimination. The results also indicated that the denture liners preserved their mechanical property after the maximum contact time with the PHMGH solution at the highest tested concentration. All PHMGH concentrations showed antifungal activity against a mature biofilm of *C. albicans* developed on denture liner surfaces. Moreover, the tensile strength was not affected by immersion in PHMGH solutions in any of the immersion periods.

Polyhexamethylene guanidine hydrochloride (PHMGH) is an organic compound from the guanidine family with cationic charge, presenting characteristics as odorless, colorless and has high solubility in water as previously mentioned [24]. In the dentistry field, it has been already tested at low concentration (0.04%) as a mouthwash to reduce oral bacterial counts and to inhibit biofilm growth [28]. Other studies

presented similar compounds for the disinfection of surfaces, contact lens and water purification [21,25,28]. Furthermore, PHMGH solution with distilled water has already shown the antimicrobial effect at 0.52% against *Bacillus subtillis* spores after 90 s of exposition and at 0.36% after 3 min [25]. An expressive antibacterial activity against Gram-positive and Gram-negative bacteria [24] is observed at low concentrations of PHMGH. A similar bacterial reduction effect is also observed after short periods of contact with PHMGH. The robust antibacterial endpoint of PHMGH [25] is attributed to the electrostatic interaction between cationic PHMGH and bacteria wall. This interaction leads to cellular disruption, leakage of intracellular components and cytosol coagulation [24]. Previous studies tested the effect of PHMGH or its derivatives against biofilm formation of *Escherichia coli* and *Staphylococcus aureus* on the surface of polyhydroxybutyrate and polylactide [29], *Streptococcus mutans* biofilm on methacrylate resin for sealant formulation [26], *Staphylococcus aureus* biofilm on polyacrylonitrile nanofibrous membranes [30]. However, to the best of our knowledge, there are no studies about the effects of PHMGH against biofilm of *Candida albicans*.

The mechanism of action against *Candida albicans* was deeply investigated in the previous report [22]. The mechanism of action for PHMGH was investigated and linked to the cationic charge and hydrophobicity. The resulting detrimental effect were: changes in the size and granularity of *Candida albicans*, besides inducing loss of phospholipid area in the membrane by forming large pores (2.3–3.3 nm) in its structure. Furthermore, potassium leakage was confirmed, suggesting membrane depolarization [22]. The present study tested after 5 and 10 min of immersion periods considering the higher surface roughness of denture liner compared to a glass surface. Additionally, this period of immersion was used because it is considered suitable for clinical use. Treatment with PHMGH water solution with 5 and 10 min of immersion was considered to simulate chemical cleaning protocols. Others studies presented similar immersion time as NaOCl (2%) for 15 min [31]; NaOCl (0.5%) and a commercial denture cleaner (Blend-A-Dent; Procter & Gamble, Schwalbach, Germany) for 3, 10 or 15 min [32].

The mean surface roughness of liners is around 3.8 μm, whereas the acrylic resin denture base material surface roughness is 2.07 μm [33]. The increased surface roughness of soft-lining materials compared to acrylic resin assists *C. albicans* adherence to the denture face that is in contact with the palatal surface [34]. The presence of *C. albicans* in denture may be a risk factor for the development of severe infections such as nosocomial pneumonia (NP) and ventilator-associated pneumonia (VAP), usually associated with UTI patients [9] which can lead to the death of patients in worst scenarios. The incidence of denture stomatitis is higher among elderly denture users, usually because of the lower motricity levels compared to younger adults [16,35]. Furthermore, to brush with dentifrice can cause damage and wear on denture liner with the increase of surface roughness [36]. From there, good chemical disinfection is essential to maintain oral and general health.

In our study, the immersion in all PHMGH groups after 10 min of immersion and 0.50% PHMGH after only 5 min presented no *C. albicans* colonies. PHMGH shows strong antimicrobial activity; however, toxicological studies suggested an association with pulmonary fibrosis after people were exposed to it in humidifier disinfectants [37]. Therefore, despite the fact that it is not the scope of this study to evaluate the cytotoxicity of PHMGH, this effect should be investigated in future investigations.

Tensile strength defines the maximum tensile stress resistance of denture liner material until rupture during use and maintenance [38]. In this study, the tensile strength was not referred to in ISO 10139-1 and ISO 10139-2, so ASTM D638-02a [27] was used due to standard test methods for tensile properties of nonrigid plastics, type IV, as well as in another study [38]. Immersion in PHMGH solution did not change the tensile strength of denture liner regardless of the concentration (Control, 0.125%, 0.25% and 0.5% of PHMGH), and immersion time applied (5 and 10 min). Other studies showed no difference in immediate weight loss, superficial roughness and tensile bond strength [39], on the

other hand, the denture liner specimens immersed in water have shown increased tensile strength than those immersed in water with denture cleaning tablets containing sodium perborate monohydrate (Clinsodent) during immediately, 24 h and seven days [19]. As limitation, the present study has tested tensile strength for an immediate effect with no evaluation after successive immersions. Following manufacturers' instructions, it is recommended that the patient return to the dentist to change the liner after 2–4 days after the initial treatment, and each subsequent treatment. Therefore, further investigations could address changes in mechanical properties during this period.

5. Conclusion

A PHMGH-water base solution was investigated for the first time as a denture liner disinfectant. The PHMGH solution was able to reach total fungal elimination of 72h-mature *Candida albicans* biofilm within 5 min and preserved its mechanical property after the maximum contact time with the solution at the highest tested concentration.

Authors' contributions

I.M. Garcia, contributed to design, data acquisition and analysis, drafted the manuscript; S.B. Rodrigues, contributed to design, data acquisition and analysis, drafted the manuscript; M.E.R. Gama, contributed to design, data acquisition, and analysis; V.C.B. Leitune, contributed to design, data analysis and data interpretation; M.A. Melo, contributed to data analysis, data interpretation, drafted the manuscript; F.M. Collares, contributed to conception, design, data acquisition, analysis and interpretation, drafted and critically revised the manuscript. All authors gave final approval and agreed to be accountable for all aspects of the work.

Declaration of competing interest

None.

References

[1] S. Patil, R.S. Rao, B. Majumdar, S. Anil, Clinical appearance of oral Candida infection and therapeutic strategies, Front. Microbiol. 6 (2015) 1391.

[2] Howard F. Jenkinson, L.Julia Douglas, KA Brogden, JM Guthmiller (Eds.), Polymicrobial Diseases Chapter 18 Interactions between Candida Species and Bacteria in Mixed Infections. Polymicrobial Diseases, ASM Press, Washington (DC), 2002.

[3] A.D. Serefko, E.J. Poleszak, A. Malm, Candida albicans denture biofilm and its clinical significance, Pol. J. Microbiol. 61 (2012) 161–167.

[4] K.R. Pachava, L.K. Nadendla, L.S. Alluri, H. Tahseen, N.P. Sajja, Invitro antifungal evaluation of denture soft liner incorporated with tea tree oil: a new therapeutic approach towards denture stomatitis, J. Clin. Diagn. Res. 9 (2015) ZC62–ZC64.

[5] M.M. Silva, E.G. Mima, A.L. Colombo, P.V. Sanita, J.H. Jorge, E.M. Massucato, et al., Comparison of denture microwave disinfection and conventional antifungal therapy in the treatment of denture stomatitis: a randomized clinical study, Oral Surg. Oral Med. Oral Pathol. Oral Radiol. 114 (2012) 469–479.

[6] E.G. Mima, C.E. Vergani, A.L. Machado, E.M. Massucato, A.L. Colombo, V.S. Bagnato, et al., Comparison of Photodynamic Therapy versus conventional antifungal therapy for the treatment of denture stomatitis: a randomized clinical trial, Clin. Microbiol. Infect. 18 (2012) E380–E388.

[7] B.A. Evren, A. Uludamar, U. Iseri, Y.K. Ozkan, The association between socio-economic status, oral hygiene practice, denture stomatitis and oral status in elderly people living different residential homes, Arch. Gerontol. Geriatr. 53 (2011) 252–257.

[8] J. Barbeau, J. Seguin, J.P. Goulet, L. de Koninck, S.L. Avon, B. Lalonde, et al., Reassessing the presence of Candida albicans in denture-related stomatitis, Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 95 (2003) 51–59.

[9] M. Wilke, R. Grube, Update on management options in the treatment of nosocomial and ventilator assisted pneumonia: review of actual guidelines and economic aspects of therapy, Infect. Drug Resist. 7 (2013) 1–7.

[10] W. Santawisuk, W. Kanchanavasita, C. Sirisinha, C. Harnirattisai, Mechanical properties of experimental silicone soft lining materials, Dent. Mater. J. 32 (2013) 970–975.

[11] M. Gundogdu, Z. Yesil Duymus, M. Alkurt, Effect of surface treatments on the bond strength of soft denture lining materials to an acrylic resin denture base, J. Prosthet. Dent 112 (2014) 964–971.

[12] A. Mese, K.G. Guzel, Effect of storage duration on the hardness and tensile bond

strength of silicone- and acrylic resin-based resilient denture liners to a processed denture base acrylic resin, J. Prosthet. Dent 99 (2008) 153–159.

[13] S. Krishnamurthy, R.B. Hallikerimath, An in-vitro evaluation of retention, colonization and penetration of commonly used denture lining materials by Candida albicans, J. Clin. Diagn. Res. 10 (2016) ZC84–Z88.

[14] G. Chladek, A. Mertas, I. Barszczewska-Rybarek, T. Nalewajek, J. Zmudzki, W. Krol, et al., Antifungal activity of denture soft lining material modified by silver nanoparticles-a pilot study, Int. J. Mol. Sci. 12 (2011) 4735–4744.

[15] S.K. Park, Y.K. Lee, B.S. Lim, C.W. Kim, Changes in properties of short-term-use soft liners after thermocycling, J. Oral Rehabil. 31 (2004) 717–724.

[16] J.A. Skupien, F. Valentini, N. Boscato, T. Pereira-Cenci, Prevention and treatment of Candida colonization on denture liners: a systematic review, J. Prosthet. Dent 110 (2013) 356–362.

[17] F. Kawano, E.R. Dootz, A. Koran 3rd, R.G. Craig, Comparison of bond strength of six soft denture liners to denture base resin, J. Prosthet. Dent 68 (1992) 368–371.

[18] L. Atar-Froyman, A. Sharon, E.I. Weiss, Y. Houri-Haddad, D. Kesler-Shvero, A.J. Domb, et al., Anti-biofilm properties of wound dressing incorporating nonrelease polycationic antimicrobials, Biomaterials 46 (2015) 141–148.

[19] D.A. Huddar, M.N. Hombesh, B. Sandhyarani, G.S. Chandu, G.S. Nanjannawar, R. Shetty, Effect of denture cleanser on weight, surface roughness and tensile bond strength of two resilient denture liners, J. Contemp. Dent. Pract. 13 (2012) 607–611.

[20] R. Brozek, R. Koczorowski, R. Rogalewicz, A. Voelkel, B. Czarnecka, J.W. Nicholson, Effect of denture cleansers on chemical and mechanical behavior of selected soft lining materials, Dent. Mater. 27 (2011) 281–290.

[21] O.A.N. Aviv, N. Laout, S. Ratner, A. Basu, A.J. Domb, Poly(hexamethylene guanidine)-poly(ethylene glycol) solid blend for water microbial deactivation, Polym. Degrad. Stabil. 129 (2016) 239–245.

[22] H. Choi, K.J. Kim, D.G. Lee, Antifungal activity of the cationic antimicrobial polymer-polyhexamethylene guanidine hydrochloride and its mode of action, Fungal Biol. 121 (2017) 53–60.

[23] Z. Zhou, D. Wei, Y. Lu, Polyhexamethylene guanidine hydrochloride shows bactericidal advantages over chlorhexidine digluconate against ESKAPE bacteria, Biotechnol. Appl. Biochem. 62 (2015) 268–274.

[24] M.K. Oule, R. Azinwi, A.M. Bernier, T. Kablan, A.M. Maupertuis, S. Mauler, et al., Polyhexamethylene guanidine hydrochloride-based disinfectant: a novel tool to fight meticillin-resistant Staphylococcus aureus and nosocomial infections, J. Med. Microbiol. 57 (2008) 1523–1528.

[25] M.K. Oule, K. Quinn, M. Dickman, A.M. Bernier, S. Rondeau, D. De Moissac, et al., Akwaton, polyhexamethylene-guanidine hydrochloride-based sporicidal disinfectant: a novel tool to fight bacterial spores and nosocomial infections, J. Med. Microbiol. 61 (2012) 1421–1427.

[26] I.M. Garcia, S.B. Rodrigues, V.C.B. Leitune, F.M. Collares, Antibacterial, chemical and physical properties of sealants with polyhexamethylene guanidine hydrochloride, Braz. Oral Res. 33 (2019) e019.

[27] A. International, ASTM D638 - 02a, Standard Test Method for Tensile Properties of Plastics, vol. 14, (2002).

[28] M. Rosin, A. Welk, O. Bernhardt, M. Ruhnau, F.A. Pitten, T. Kocher, et al., Effect of a polyhexamethylene biguanide mouthrinse on bacterial counts and plaque, J. Clin. Periodontol. 28 (2001) 1121–1126.

[29] M. Walczak, M.S. Brzezinska, A. Richert, A. Kalwasińska, The effect of polyhexamethylene guanidine hydrochloride on biofilm formation on polylactide and polyhydroxybutyrate composites, Int. Biodeterior. Biodegrad. 98 (2015) 1–5.

[30] C.Y.X.L. Yan Mei, A simple approach to constructing antibacterial and anti-biofouling nanofibrous membranes, Biofouling 30 (2014) 313–322.

[31] S. Hahnel, M. Rosentritt, R. Burgers, G. Handel, R. Lang, Candida albicans biofilm formation on soft denture liners and efficacy of cleaning protocols, Gerodontology 29 (2012) e383–e391.

[32] M.A. Ferreira, T. Pereira-Cenci, L.M. Rodrigues de Vasconcelos, R.C. Rodrigues-Garcia, A.A. Del Bel Cury, Efficacy of denture cleansers on denture liners contaminated with Candida species, Clin. Oral Invest. 13 (2009) 237–242.

[33] P.L. Madeira, L.T. Carvalho, M.A. Paschoal, E.M. de Sousa, E.B. Moffa, M.A. da Silva, et al., In vitro effects of lemongrass extract on Candida albicans biofilms, human cells viability, and denture surface, Front. Cell Infect. Microbiol. 6 (2016) 71.

[34] E.U. Nevzatoglu, M. Ozcan, Y. Kulak-Ozkan, T. Kadir, Adherence of Candida albicans to denture base acrylics and silicone-based resilient liner materials with different surface finishes, Clin. Oral Invest. 11 (2007) 231–236.

[35] A.Z. Yildirim-Bicer, I. Peker, G. Akca, I. Celik, In vitro antifungal evaluation of seven different disinfectants on acrylic resins, BioMed Res. Int. 2014 (2014) 519098.

[36] M.M. Badaro, T.P. Prates, V.M.F. Leite-Fernandes, V.C. Oliveira, H.F.O. Paranhos, C.H. Silva-Lovato, In vitro evaluation of resilient liner after brushing with conventional and experimental Ricinus communis-based dentifrices, J. Prosthodont. 28 (2019) e857–e862.

[37] K. Park, An analysis of a humidifier disinfectant case from a toxicological perspective, Environ. Health Toxicol. 31 (2016) e2016013.

[38] S. Oguz, M.M. Mutluay, O.M. Dogan, B. Bek, Effect of thermocycling on tensile strength and tear resistance of four soft denture liners, Dent. Mater. J. 26 (2007) 296–302.

[39] R.M. Garcia, B.T. Leon, V.B. Oliveira, A.A. Del Bel Cury, Effect of a denture cleanser on weight, surface roughness, and tensile bond strength of two resilient denture liners, J. Prosthet. Dent 89 (2003) 489–494.