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Original article

2-methacryloyloxyethyl phosphorylcholine polymer treatment prevents *Candida albicans* biofilm formation on acrylic resin

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Abstract

Purpose: We aimed to evaluate the effectiveness of photoreactive 2-methacryloyloxyethyl phosphorylcholine (MPC) in inhibiting *Candida albicans* biofilm formation on polymethyl methacrylate (PMMA) and assess its mechanism and need for re-application by evaluating its interaction with salivary mucin and durability during temperature changes.

Methods: PMMA discs were used as specimens. The MPC coating was applied using the spray and cure technique for the treatment groups, whereas no coating was applied to the control. The MPC treatment (MT) groups were further differentiated based on the number of thermal cycles involved (0, 1000, 2500, and 5000). The optical density was measured to assess mucin adsorption (MA). Contact angle (CA) was calculated to evaluate surface hydrophilicity. The presence of MPC components on the PMMA surface was assessed using X-ray photoelectron spectroscopy (XPS). *C. albicans* biofilms were evaluated qualitatively (scanning electron microscope images) and quantitatively (colony-forming units (CFUs)). Statistical analysis was conducted using two-way analysis of variance and Tukey's multiple comparison test.

Results: MA rate and CA increased significantly in the MT groups, which exhibited significantly fewer CFUs and thinner biofilms than those of the control group. Based on the XPS, MA, and CFU evaluations, the durability and efficacy of the MPC coating were considered stable up to 2500 thermal cycles. Additionally, a significant interaction was observed between mucin concentration and MPC efficacy.

Conclusions: The photoreactive MPC coating, which was resistant to temperature changes for approximately 3 months, effectively prevented *C. albicans* biofilm formation by modifying surface hydrophilicity and increasing mucin adsorption.

Keywords: *Candida albicans*, Denture plaque, 2-methacryloyloxyethyl phosphorylcholine, PMMA

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1. Introduction

Polymethyl methacrylate (PMMA), or acrylic resin, is commonly used in medical and dental devices, including dentures[1,2]. This material possesses certain advantages, such as easy manipulation and low manufacturing costs. However, it also has several properties such as porosity, hydrophobicity, and high water absorption that contribute to microbial adhesion[3–5]. Microbial adhesion results in biofilm formation, in which the extracellular polymeric substances produced by microorganisms act as barriers that prevent antiseptics from penetrating the cells[6]. These phenomena are often observed in resin devices that interact with external environments.

Candidiasis is a typical opportunistic infection caused by abnormalities in the local or systemic defense mechanisms of older people, which tends to recur and become intractable owing to the weakened immune system of the older people[7]. *Candida* spp. co-aggregate with other pathogenic bacteria and cause systemic diseases. They form a strong biofilm; hence, its removal from resin materials, such as dentures, ureteral catheters, and gastrotomy tubes, is important[8]. Furthermore, *Candida albicans* is recognized as a major opportunistic oral pathogen that causes the most common infection of denture wearers, denture stomatitis, owing to its affinity for acrylic resin and dimorphic features[3]. Countermeasures have been implemented through two strategies: irrigating devices with appropriate antiseptic drugs or improving the anti-biofouling properties of the material surface[9]. Various methods have been introduced to improve the anti-biofouling properties of acrylic resins, such as incorporation of nanoparticles into the matrix, antifungal thin-film polymers, surface property modification, and 2-methacryloyloxyethyl phosphorylcholine (MPC) coating[2,3,10].

MPC is a highly hydrophilic material used to coat medical de-

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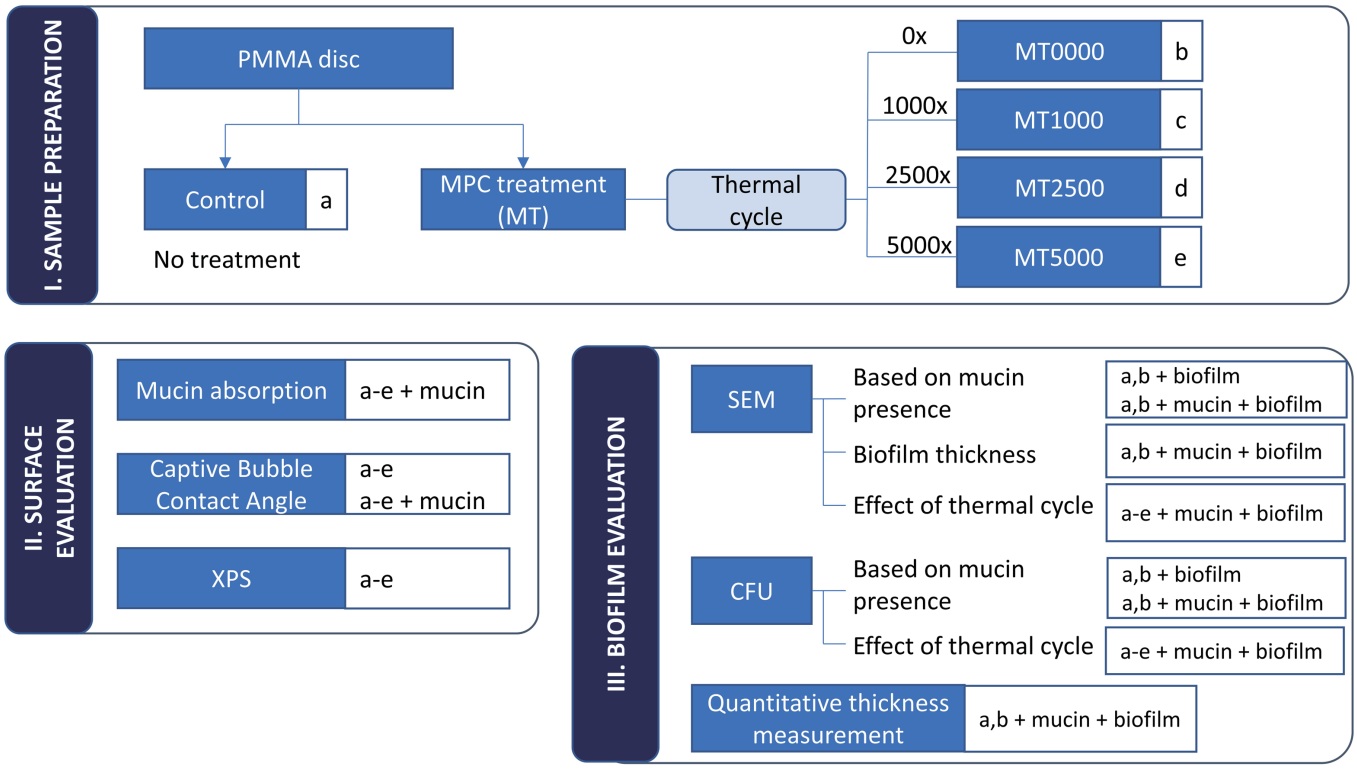

Fig. 1. Research flow diagram of this study. Sample classifications into five groups are coded with a, b, c, d, and e to further describe the comparisons conducted in various evaluations. PMMA: polymethyl methacrylate, MPC: 2-methacryloyloxyethyl phosphorylcholine, XPS: X-ray photoelectron spectroscopy, SEM: scanning electron microscope, CFU: colony-forming unit.

vices; it can substantially prevent cell adhesion by reducing protein absorption[11]. Additionally, MPC as a coating material is highly biocompatible and completely harmless and has already been approved by the Food and Drug Administration in the United States and the Ministry of Health and Labor Welfare in Japan[10,12]. It has been widely used as a coating material in cardiovascular devices, ophthalmic devices, and artificial hip joints[10]. MPC possesses anti-inflammatory properties and protects oral epithelial cells[13], clinical trial have been performed evaluating its efficacy as a mouthwash[14]. Additionally, MPC polymers have been used to modify PMMA[11,15–20].

However, MPC polymers do not form strong bonds with PMMA. A new method, photocurable co-polymerization with n-butyl methacrylate (BMA) and 2-methacryloyloxyethyl-4-azidobenzoate (MPAz) formed poly (MPC-co-BMA-co-MPAz) (PMBPAz), resulting in the formation of a stronger bond with the PMMA surface than MPC polymers formed with PMMA [15]. Further improvement resulted in pre-mixed PMBPAz in a sprayable solution, easing the MPC coating process in chairside treatment settings without complicated armamentarium. Previous studies have evaluated the efficacy of MPC coating on PMMA in preventing protein absorption[11], *Streptococcus mutans*[16], clinical denture plaque at 2 weeks[15,17], *C. albicans* at the initial adhesion phase[18], and in reducing bacterial quantity while maintaining homeostasis[19]. No studies till date have reported the efficacy of the innovative MPC coating on acrylic resin in preventing mature *C. albicans* biofilm formation and the durability of PMBPAz coating developed using this method.

When a denture is placed in the oral cavity, its surface immediately contacts the saliva in the surrounding environment, which further covers the denture surface to form a pellicle. Mucin, one of the major components found in denture pellicles, possesses several biological properties in terms of interaction with microorganisms, which may promote adhesion of certain microorganisms[20], conversely, reduce adhesion by agglomerating cells for easier clearance, or suppress virulence, such as hyphae formation of *C. albicans*[21,22]. Currently, the mucin feature that would be exhibited when this MPC coating is applied on the PMMA surface has not yet been identified.

This study aimed to evaluate the effectiveness of a simple spray and cure system for PMBPAz coating by observing *C. albicans* biofilm formation on PMMA. In addition, we analyzed the interaction of this coating material with salivary mucin and its resistance to temperature changes under intraoral conditions to assess its durability.

2. Materials and Methods

The research flow diagram of this study is briefly illustrated in **Figure 1**, with the following procedural details:

2.1. Specimen preparation

2.1.1. Base specimen and MPC coating

The base specimens were made of heat-cured PMMA dentures (ACRON®, GC Corporation, Tokyo, Japan) according to the manufacturer's instructions. The specimens were 2 mm thick discs with a diameter of 12 mm. Following heat polymerization, the discs were manually polished using waterproof sandpapers (#1200 and #2400). Base specimens without any treatment were assigned to the control group.

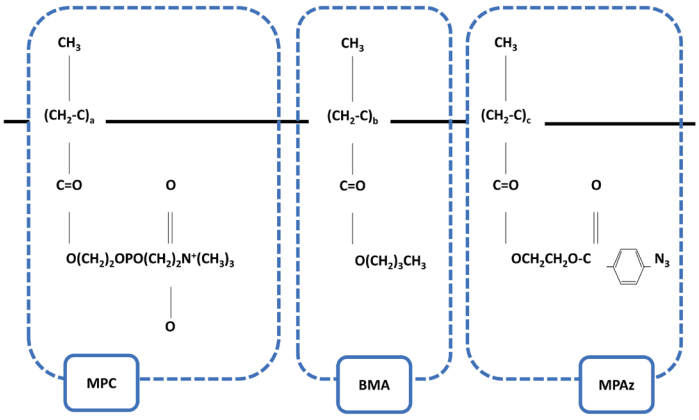

Fig. 2. Structure of photopolymerized MPC-co-BMA-co-MPAz (PMBPAz) polymer used in this study. MPC: 2-methacryloyloxyethyl phosphorylcholine, BMA: n-butyl methacrylate, MPAz: 2-methacryloyloxyethyl-4-azidobenzoate.

The base specimens were coated with MPC using the following procedure: a photoreactive PMBPAz polymer (**Fig. 2**) dissolved in ethanol in the form of a liquid spray (Kirei Keep®, Sun Medical Company, Moriyama, Japan) was applied to the specimen surface, followed by air-drying for 30 s. Polymerization was performed using 260–280 nm UV light inside a curing box (Kirei Keep®, Sun Medical Company, Moriyama, Japan) for 3 min. Finally, specimens were washed with distilled water and stored in a desiccator.

2.1.2. Durability test

The MPC-coated specimens were soaked overnight in distilled water and then transferred to a thermal cycling machine (K178; Tokyo Giken Inc., Tokyo, Japan). The thermal cycling procedure was performed between 5°C and 55°C for 1000 cycles (MT1000 group), 2500 cycles (MT2500 group), and 5000 cycles (MT5000 group) to simulate temperature changes inside the oral cavity. Henceforth, the term MPC-treated specimens is referred to as MPC treatment (MT), followed by four digits (e.g., MT0000 = without thermal cycle) representing the number of thermal cycles that has been used to treat it.

The number of thermal cycles applied herein was based on a previous study suggesting that 10,000 cycles represent 1 year of intraoral thermal changes[23].

2.2. Surface evaluation

2.2.1. Mucin adsorption

The control, MPC-treated, and thermal cycled specimens were immersed in 0.5 mg/mL mucin (Type I Bovine Submaxillary Mucin, Sigma Aldrich, Missouri, USA) in phosphate-buffered saline (PBS) for 4 h on a 70 rpm shaker at 37°C, followed by washing with PBS twice. The amount of mucin adsorbed by each group was evaluated using a mucin adsorption assay. It was performed by applying 2.5% Alcian blue solution (Wako, Fujifilm, Osaka, Japan) for 10 min, washing with PBS three times, and collecting with 1 ml of 30% H_2O_2 to be scanned using a plate reader (Bio-Rad iMark™, California, USA) at an optical density (OD) of 595 nm.

2.2.2. Captive bubble contact angle

The captive bubble contact angles of the specimens were measured in a glass vessel filled with distilled water. Each specimen was inverted in a glass vessel such that the upper surface faced the base of the glass vessel. After 5 min, a 1 mL air bubble was deployed below the surface of the specimens using a U-shaped needle. Images of the interface between the air bubble and specimen surface were captured using a microscope (USB microscope M2, Scalar Corporation, Tokyo, Japan). Contact angle measurements were performed using the ImageJ software (National Institutes of Health, Maryland, USA). A high contact angle indicates a hydrophilic surface in this test.

2.2.3. X-ray photoelectron spectrophotometer (XPS)

The surface elemental composition of the PMMA coated with MPC was analyzed using XPS (PHI5000 VersaProbe II, ULVAC-PHI Inc., Kanagawa, Japan) equipped with a 15 kV, Al Kα monochromatic radiation source at the anode. The photoelectron releasing source energy was 1486.6 eV, with a 100 μm beam diameter and 45° inclination toward the analyzer. The signals of the characteristic elements phosphorus and nitrogen were evaluated at 134 and 403 eV, respectively.

2.3. Evaluation of *C. albicans* biofilm formation

2.3.1. Biofilm formation

A clinical isolate of *C. albicans* from a denture plaque suspension was obtained by centrifugation of *C. albicans* cultured in Sabouraud dextrose medium aerobically at 37°C for 48 h. After coating with 0.5 mg/mL mucin, the specimens were placed in a 24-well plate. A drop of *C. albicans* suspension (20 μL) was placed on the resin disc for 5 min, followed by the addition of 2 mL YNBNP (yeast nitrogen base, N-acetylglucosamine, phosphate) medium supplemented with 10% fetal bovine serum (Gibco, Thermo Fischer Scientific, Massachusetts, USA). The biofilm was incubated aerobically at 37°C for 72 h.

2.3.2. Observation of *C. albicans* biofilm

After incubation, the specimens were washed twice with PBS, followed by fixation in 2.5% glutaraldehyde solution for 15 min. Serial dehydration was performed after fixation with 40, 75, and 99% ethanol. Gold coating was applied to the specimens using an ion coater (IB-3, Eiko Corporation, Tokyo, Japan) at 3 mA for 5 min. Finally, biofilm morphology was observed using a low-vacuum scanning electron microscope (SEM) (Miniscope TM1000®, Hitachi, Japan).

To observe the biofilm thickness, specimens with biofilm underwent fixation and dehydration, as mentioned above, and were subsequently soaked in a 2-hydroxyethyl methacrylate solution (Technovit®7100, Kulzer Technik, Wehrheim, Germany) for 30 min before conditioning. The specimens were then placed in a mold and embedded in histological cold-curing resin (Technovit®7100, Kulzer Technik, Wehrheim, Germany). Embedded specimens were cut in the sagittal plane using a microtome, followed by overnight polymerization. Before cross-sectional SEM examination, the specimens were coated with the electron conductive medium, TI Blue (3% platinum blue; Nissin EM, Tokyo, Japan), for 20 min at room temperature (22 ± 2°C), rinsed with distilled water, and air dried. Biofilm thickness was measured by determining the lowest point of the biofilm surface to obtain a horizontal line parallel to the PMMA surface. Finally, micrometer-scale thickness measurement was performed using a digital stereomicroscope software (VHX-F, Keyence, Osaka, Japan).

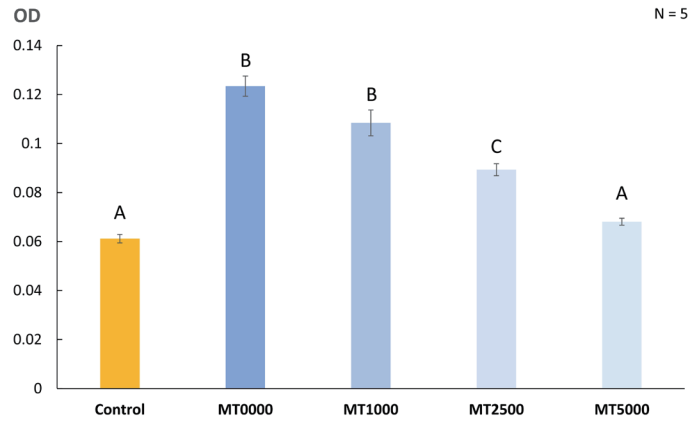

Fig. 3. Comparison of adsorbed mucin amounts on the polymethyl methacrylate surface in the five groups. * Different alphabets on the bar represents a significant difference. MT: 2-methacryloyloxyethyl phosphorylcholine treatment.

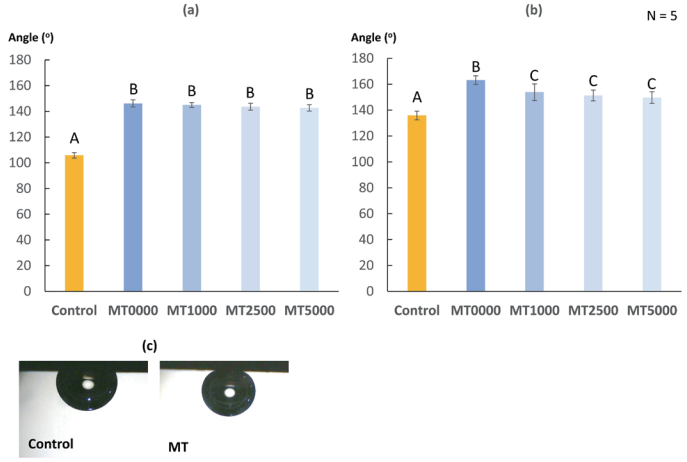

Fig. 4. Captive bubble contact angles of the five groups without mucin treatment (a) and with mucin treatment (b); and the image of contact interface between air bubble and polymethyl methacrylate surface inside water (c). MT: 2-methacryloyloxyethyl phosphorylcholine treatment *Different alphabets on the bar represents a significant difference. **High contact angle represents hydrophilic properties in captive bubble test.

2.3.3. Quantification of biofilm cells

The resin specimens were removed from the biofilm growth medium and washed twice with PBS to remove any unattached cells. The attached cells on the resin surface were dissolved in 1 mL of 0.25% trypsin in saline solution by pipetting for 2 min. A 10,000 × serial dilution of the solution was prepared and plated on Sabouraud dextrose agar. After 24 h of incubation, the viable cell numbers of the biofilms were quantified based on colony forming units (CFU).

2.4. Statistical analysis

The SPSS software (version 25, SPSS Japan Inc., Tokyo, Japan) was used for statistical analyses. The data collected for each variable were analyzed using one-way analysis of variance (ANOVA) with Tukey's multiple comparison test at a *P*-value of 0.05. In addition, a two-way ANOVA was performed to observe the interaction between mucin adsorbance and MPC coating based on the contact angle and CFU values.

3. Results

3.1. Mucin adsorption

The amount of mucin adsorbed on each specimen surface was determined by the OD of Alcian Blue (**Fig. 3**). The value for the MT0000 group before thermal cycling was significantly high, approximately twice that of the control group. The values gradually decreased with repeated thermal cycles, and the value for the MT5000 group was close to that of the control.

3.2. Contact angle

Figure 4 shows the captive bubble contact angles of the five groups with and without mucin treatment. The contact angle of the control group without mucin treatment was only 105.8 ± 2.2°, while those of the MT0000, MT1000, MT2500, and MT5000 groups were 146.2 ± 2.8°, 145.0 ± 1.9°, 143.6 ± 2.7°, and 142.73 ± 2.5°, respectively. The surface hydrophilicity of all MPC treatment groups improved significantly ($P < 0.05$). Thermal cycling did not affect the contact angle.

The contact angles of the specimens with mucin treatment were significantly higher than those of the specimens without mucin treatment under all conditions ($P < 0.05$). However, there was also a significant reduction in the contact angle values in the thermally cycled groups compared to those in the MT0000 group. A significant interaction was observed between mucin and MPC treatment with respect to contact angle values ($P < 0.05$).

3.3. XPS

Surface elemental composition analysis was performed to confirm the presence of the PMBPAz coating on the PMMA surface and evaluate the coating layer changes following the thermal cycling procedure. The XPS signals for phosphorus (phosphate group–P–O) and nitrogen (ammonium group–$N^+(CH_3)_3$), which represent the PMBPAz molecules, were evaluated (**Fig. 5**). The ammonium and phosphate group profiles were similar in the MT0000, MT1000, and MT2500 groups, whereas an approximately half peak height reduction was observed in the MT5000 group, and no peak was observed in the control group. The MPC component concentrations on the PMMA surface were stable after 2500 thermal cycles; however, a notable reduction was observed after 5000 thermal cycles.

3.4. Scanning electron micrograph

Figure 6 shows the comparison of *C. albicans* biofilm morphology between the control and MT0000, with and without mucin, based on 2000× magnification SEM images. The dominant cell type observed in the biofilm SEM images was round yeast cells along with hyphae and pseudo-hyphae. Furthermore, a notable difference was observed in the MPC treated resin with mucin, where the biofilm was thin and the PMMA surface was visible.

Figure 7 displays sagittal section images of the biofilm to comprehensively confirm the influence of MPC treatment on biofilm morphology thickness. The biofilm thickness of the control group (65 ± 20 μm) was approximately twice of that of the MT0000 group (34 ±

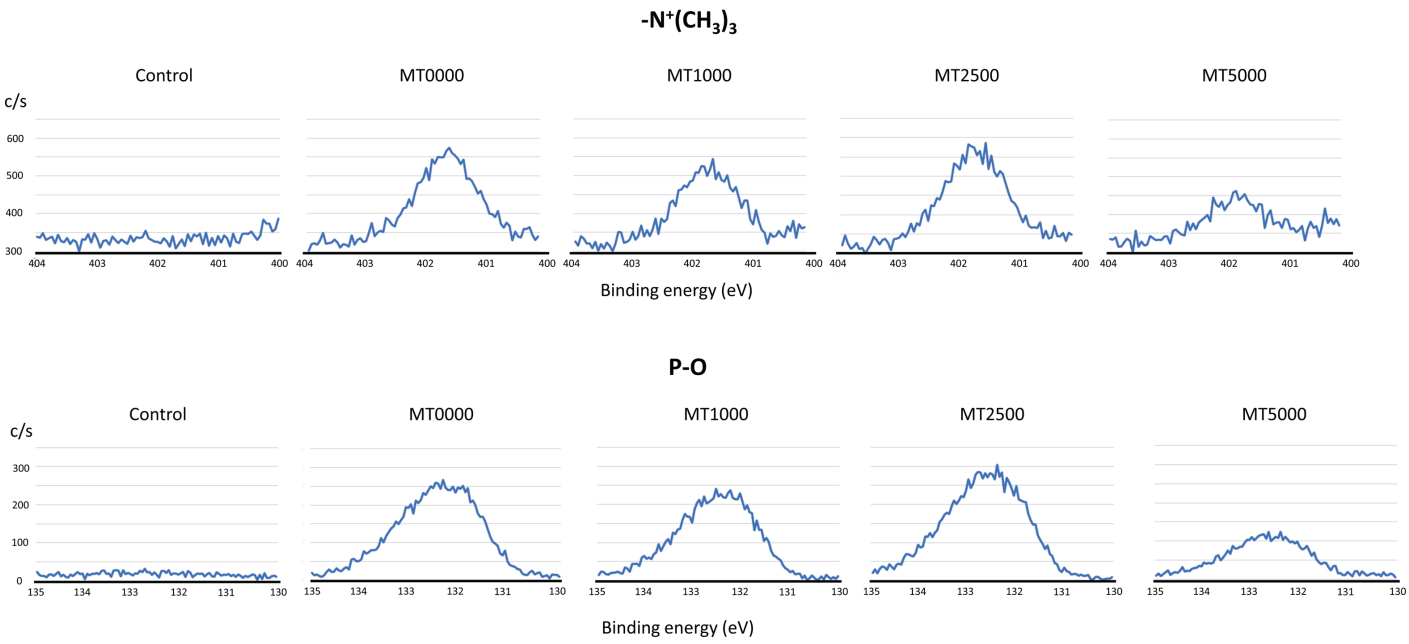

Fig. 5. Results of X-ray Photoelectron Spectroscopy confirming the availability of 2-methacryloyloxyethyl phosphorylcholine (MPC) components on the polymethyl methacrylate surface. MT: MPC treatment.

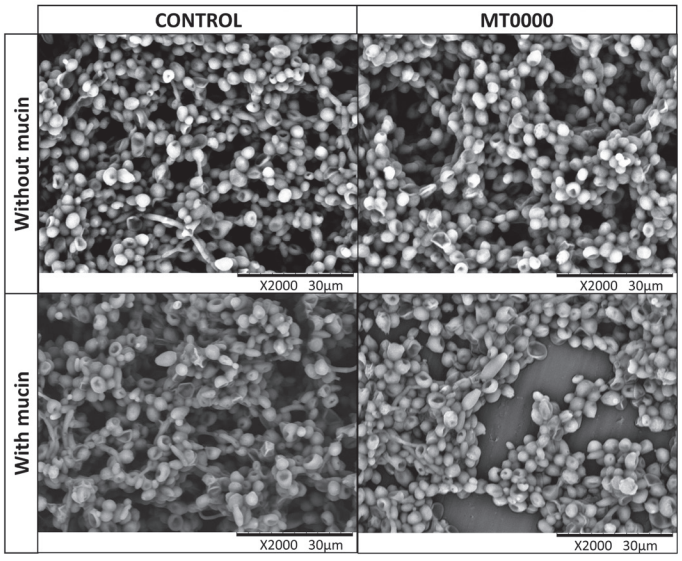

Fig. 6. Scanning electron micrograph images of *C. albicans* biofilms on the specimens with/without mucin and 2-methacryloyloxyethyl phosphorylcholine treatments (before thermal cycling)

7 μm) with a statistical difference ($P<0.05$).

Figure 8 shows SEM images of the biofilm morphology before and after thermal cycling. As shown in **Figure 6**, a thick stratified biofilm, which presented mostly round yeast cells, including pseudo-hyphae and hyphae cells, was observed on the control specimen. A thin *Candida* biofilm, in which the resin surface area was directly visible, was found in the MPC group before thermal cycling (MT0000). The *Candida* biofilms on MT1000 and MT2500 were similar to those on MT0000, whereas the surface area covered with the *Candida* biofilm gradually increased with repeated thermal cycles. *Candida* biofilms on MT5000 were thick and similar to those of the control.

3.5. Colony-forming units

Figure 9 shows the CFUs of *C. albicans* biofilms formed on the specimens before and after MPC treatment. The CFUs of the MPC-coated specimens were significantly lower than those of the non-coated specimens. Mucin treatment improved the reduction rate to 60.3%, whereas only a 39.9% CFU reduction was observed for the MPC-treated specimens without mucin compared to that of the control. In addition, there was a significant interaction between mucin and MPC treatment with regard to CFU values.

Figure 10 illustrates the changes in CFU before and after thermal cycling in specimens with mucin-treated surfaces. The control specimen had the highest CFU ($3.64 \pm 0.35 \times 10^7$), which was significantly higher ($P < 0.05$) than that of the other groups. No significant difference in CFU was found among MT0000 ($1.44 \pm 0.38 \times 10^7$), MT1000 ($1.52 \pm 0.19 \times 10^7$), and MT2500 group ($1.81 \pm 0.35 \times 10^7$). However, the CFU was significantly higher ($P< 0.05$) in the MT5000 group ($2.77 \pm 0.47 \times 10^7$) than that in the other groups.

4. Discussion

The efficacy of MPC in preventing microbial adherence has been tested to explain the hypothesis that MPC coating does not inhibit cell proliferation but increases surface hydrophilicity[18,24]. PMBPAz, a new photopolymerized form of MPC that forms a stronger covalent bond with the PMMA surface than MPC and has improved durability over time, has been developed[15]. The efficacy of PMBPAz against streptococci has been evaluated[16]; however, there have been no reports on its efficacy against *Candida* spp. In addition, the durability of PMBPAz coatings has not yet been studied. When microorganisms adhere to various medical/dental devices, they also adhere to

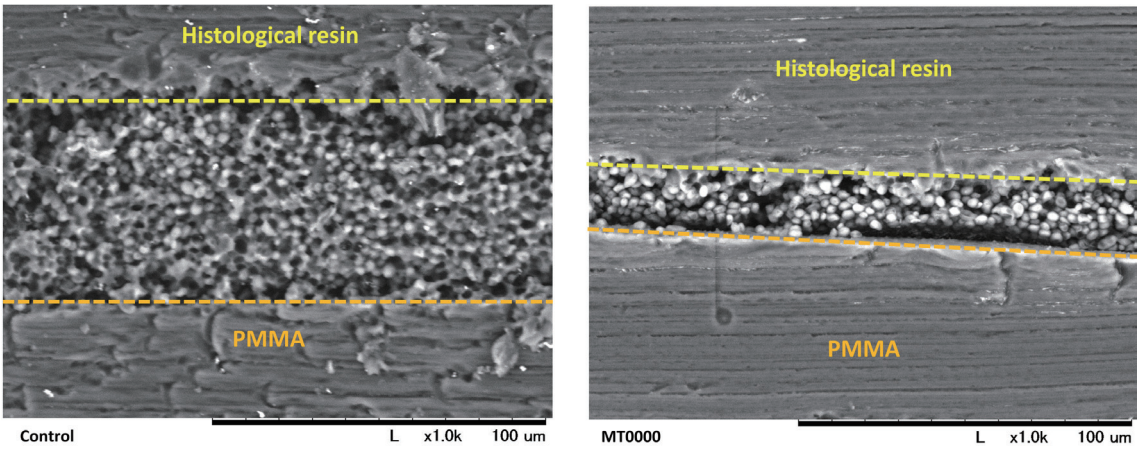

Fig. 7. Sagittal section images of *C. albicans* biofilm on the specimens with/without 2-methacryloyloxyethyl phosphorylcholine treatments (with mucin treatment and before thermal cycling). Yellow line represents the border of histological resin as a fixation media, while orange line defines the polymethyl methacrylate (PMMA) border.

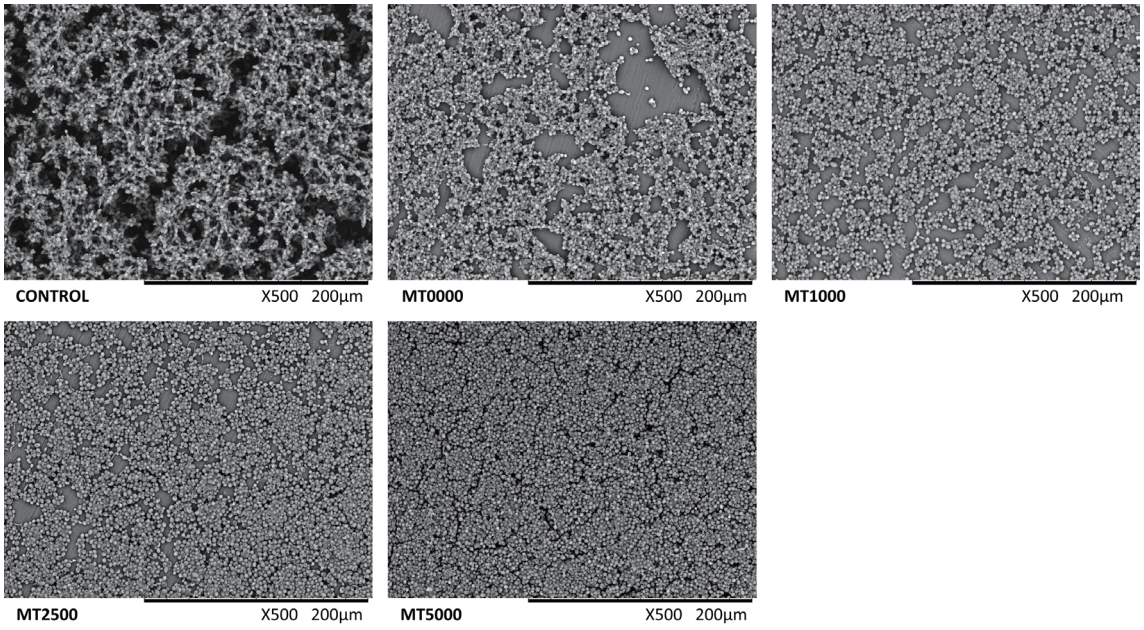

Fig. 8. Scanning Electron Micrograph images of *C. albicans* biofilm on the specimens before and after thermal cycling (with mucin treatment). MT: 2-methacryloyloxyethyl phosphorylcholine treatment.

glycoproteins on their surfaces. Salivary components, such as mucin, are typical examples of glycoprotein sources in the oral cavity[20]. Microorganism adhesion begins by securing a foothold, producing extracellular polysaccharides, and finally forming biofilms composed of microbial cell aggregates[6]. In this study, we evaluated the surface properties of the PMBPAz coating based on the contact angle, which represents the hydrophilicity and amount of mucin adsorption. These variables aided in predicting the efficacy of the PMBPAz coating properties in a clinical setting.

The MPC coating increased the number of surface functional groups and enhanced the hydrophilicity of the PMMA surface. In addition, with MPC treatment the amount of adsorbed mucin was higher than that without MPC treatment, which further increased the degree of hydrophilicity. Observation of biofilm morphology using SEM images revealed that the thickness and stratification of the biofilm decreased with MPC treatment, with a corresponding decrease in the number of CFUs. Mucin treatment increased the ability to inhibit biofilm formation in the MPC treatment group, and a significant interaction between mucin and MPC coating was observed in the two-way ANOVA analysis. The SEM morphology suggested that *C. albicans* biofilm formation was suppressed by MPC and mucin treatments. The interaction may be owing to the fact that hydrophilicity is enhanced by MPC and mucin treatment as well as by the microbial effect of the mucin itself. Although salivary mucin initially provides a glycoprotein for *C. albicans* attachment, it also prevents microbial adhesion by preventing normal flora from becoming opportunistic pathogens[21]. Yoshijima *et al.* reported that increasing the surface hydrophilicity effectively prevents yeast and hyphae adherence[25]. The mucin used in this study possesses a structure similar to that of

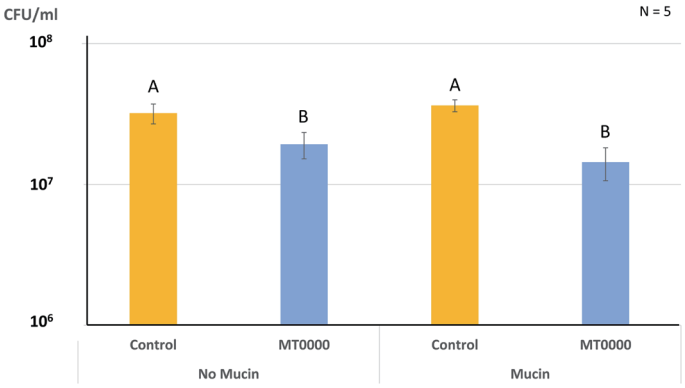

Fig. 9. Colony-forming units of *C. albicans* biofilms on the specimens with and without 2-methacryloyloxyethyl phosphorylcholine (MPC) coating as well as with and without mucin treatment. *Different alphabets on the bar represents a significant difference. MT: MPC treatment.

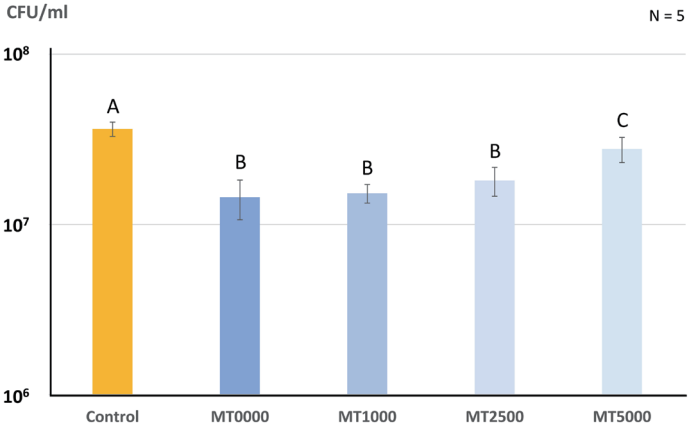

Fig. 10. Colony-forming units of *C. albicans* biofilm on the specimens before and after thermal cycling (with mucin treatment). *Different alphabets on the bar represents a significant difference. MT: 2-methacryloyloxyethyl phosphorylcholine treatment.

the MUC5B protein[26], which reduces *C. albicans* hyphae and biofilm formation without killing it[22]. Tsukahara *et al*.[19] also reported that MPC is able to reduce bacterial quantity while maintaining homeostasis without changing the bacterial composition in the oral cavity, which is similar to the salivary mucin features mentioned above.

There are no reports on the durability of PMBPAz coatings during temperature changes. The number of thermal cycles herein was based on a previous study suggesting that 10,000 cycles represent 1 year of intraoral thermal changes[24]. Thermal cycling was conducted at 1000, 2500, and 5000 cycles, as the manufacturer suggested re-application of the MPC coating every 3 months. There was no significant reduction in the MPC coating efficacy in inhibiting biofilm formation morphologically and quantitatively after 1000 and 2500 thermal cycles; however, a significant reduction was observed in the MT5000 group. This result indicates that the efficacy of the PMBPAz coating was sustained for approximately 3 months. This could be explained by the reduction in the MPC components on the surface, as shown in the XPS data, and reduced mucin adsorption as opposed to surface hydrophilicity alone.

This study has three limitations. First, the efficacy of the PMBPAz coating was evaluated only at a single time point, that is, at 3 days, which is the incubation period to determine the ability of *C. albicans* to form a mature biofilm. We speculated that the initial adhesion in the preliminary experiment and in our previous reports[18,22] was almost proportional to our present results. Second, the mechanism of biofilm formation prevention cannot be explained solely based on surface hydrophilicity and mucin adsorption. However, we found that the reduction in *Candida* biofilm was sustained by the PMBPAz coating for approximately 3 months compared to that of the uncoated PMMA surface. The interaction between the surface properties and the biological origin of glycoproteins needs to be explored. Third, these results might not always reflect the clinical situation, as there are various types of microorganisms constituting the biofilm *in vivo*, unlike this specific species in the *in vitro* biofilm. Furthermore, additional clinical durability aspects should be considered, such as mechanical and chemical cleaning and friction with foods. Further studies should investigate the involvement of mucin in enhancing the efficacy of PMBPAz coatings to validate the results of this study.

5. Conclusions

Photoreactive MPC polymer (PMBPAz) treatment of PMMA effectively prevents *C. albicans* biofilm formation. This efficacy might depend on the increased hydrophilic surface properties and the high mucin adsorption rate. In addition, during temperature changes, the PMBPAz coating was stable for approximately 3 months.

Acknowledgements

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Conflicts of interest

All authors declare no conflict of interest.

References

[1] Alla RK, Sajjan S, Alluri VR, Ginjupalli K, Upadhya N. Influence of Fiber Reinforcement on the Properties of Denture Base Resins. J Biomater Nanobiotechnol. 2013;4:91–7. https://doi.org/10.4236/jbnb.2013.41012

[2] Zafar MS. Prosthodontic Applications of Polymethyl Methacrylate (PMMA): an Update. Polymers (Basel). 2020;12:2299. https://doi.org/10.3390/polym12102299, PMID:33049984

[3] Gleiznys A, Zdanavičienė E, Žilinskas J. Candida albicans importance to denture wearers. A literature review. Stomatologija. 2015;17:54–66. PMID:26879270

[4] Fang J, Wang C, Li Y, Zhao Z, Mei L. Comparison of bacterial adhesion to dental materials of polyethylene terephthalate (PET) and polymethyl methacrylate (PMMA) using atomic force microscopy and scanning electron microscopy. Scanning. 2016;38:665–70. https://doi.org/10.1002/sca.21314, PMID:26991988

[5] Cao L, Xie X, Wang B, Weir MD, Oates TW, Xu HHK, *et al.* Protein-repellent and antibacterial effects of a novel polymethyl methacrylate resin. J Dent. 2018;79:39–45. https://doi.org/10.1016/j.jdent.2018.09.007, PMID:30248381

[6] Czaczyk K, Myszka K. Biosynthesis of extracellular polymeric substances (EPS) and its role in microbial biofilm formation. Pol J Environ Stud. 2007;6:799–806.

[7] Hirota K, Yumoto H, Sapaar B, Matsuo T, Ichikawa T, Miyake Y. Pathogenic factors in *Candida* biofilm-related infectious diseases. J Appl Microbiol. 2017;122:321–30. https://doi.org/10.1111/jam.13330, PMID:27770500

[8] Kojic EM, Darouiche RO. Candida infections of medical devices. Clin Microbiol Rev. 2004;17:255–67. https://doi.org/10.1128/CMR.17.2.255-267.2004, PMID:15084500

[9] Cavalheiro M, Teixeira MC. *Candida* Biofilms: Threats, Challenges, and Promising Strategies. Front Med (Lausanne). 2018;5:28. https://doi.org/10.3389/fmed.2018.00028, PMID:29487851

[10] Ishihara K. Revolutionary advances in 2-methacryloyloxyethyl phosphorylcholine polymers as biomaterials. J Biomed Mater Res A. 2019;107:933–43. https://doi.org/10.1002/jbm.a.36635, PMID:30701666

[11] Takahashi N, Iwasa F, Inoue Y, Morisaki H, Ishihara K, Baba K. Evaluation of the durability and antiadhesive action of 2-methacryloyloxyethyl phosphorylcholine grafting on an acrylic resin denture base material. J Prosthet Dent. 2014;112:194–203. https://doi.org/10.1016/j.prosdent.2013.08.020, PMID:24461942

[12] Iwasaki Y, Aiba Y, Morimoto N, Nakabayashi N, Ishihara K. Semi-interpenetrating polymer networks composed of biocompatible phospholipid polymer and segmented polyurethane. J Biomed Mater Res. 2000;52:701–8. https://doi.org/10.1002/1097-4636(20001215)52:4<701::AID-JBM15>3.0.CO;2-6, PMID:11033553

[13] Yumoto H, Hirota K, Hirao K, Miyazaki T, Yamamoto N, Miyamoto K, *et al.* Anti-inflammatory and protective effects of 2-methacryloyloxyethyl phosphorylcholine polymer on oral epithelial cells. J Biomed Mater Res A. 2015;103:555–63. https://doi.org/10.1002/jbm.a.35201, PMID:24753309

[14] Fujiwara N, Yumoto H, Miyamoto K, Hirota K, Nakae H, Tanaka S, *et al.* 2-Methacryloyloxyethyl phosphorylcholine (MPC)-polymer suppresses an increase of oral bacteria: a single-blind, crossover clinical trial. Clin Oral Investig. 2019;23:739–46. https://doi.org/10.1007/s00784-018-2490-2, PMID:29766378

[15] Ikeya K, Fukunishi M, Iwasa F, Inoue Y, Ishihara K, Baba K. 2-methacryloyloxyethyl phosphorylcholine polymer treatment of complete dentures to inhibit denture plaque deposition. J Vis Exp. 2016;118:e54965. https://doi.org/10.3791/54965, PMID:28060350

[16] Fukunishi M, Inoue Y, Morisaki H, Kuwata H, Ishihara K, Baba K. A Polymethyl Methacrylate–Based Acrylic Dental Resin Surface Bound with a Photoreactive Polymer Inhibits Accumulation of Bacterial Plaque. Int J Prosthodont. 2017;30:533–40. https://doi.org/10.11607/ijp.5332, PMID:29084298

[17] Ikeya K, Iwasa F, Inoue Y, Fukunishi M, Takahashi N, Ishihara K, *et al.* Inhibition of denture plaque deposition on complete dentures by 2-methacryloyloxyethyl phosphorylcholine polymer coating: A clinical study. J Prosthet Dent. 2018;119:67–74. https://doi.org/10.1016/j.prosdent.2017.02.012, PMID:28506653

[18] Fujiwara N, Murakami K, Yoshida K, Sakurai S, Kudo Y, Ozaki K, *et al.* Suppressive effects of 2-methacryloyloxyethyl phosphorylcholine (MPC)-polymer on the adherence of *Candida* species and MRSA to acrylic denture resin. Heliyon. 2020;6:e04211. https://doi.org/10.1016/j.heliyon.2020.e04211, PMID:32577575

[19] Tsukahara A, Ikeya K, Morisaki H, Iwasa F, Inoue Y, Fukunishi M, *et al.* 2-methacryloyloxyethyl phosphorylcholine polymer can facilitate the reduction in bacterial quantity while maintaining bacterial homeostasis in denture plaque. Biomed Res (Aligarh). 2020;31:118–24.

[20] Wickström C, Hamilton IR, Svensäter G. Differential metabolic activity by dental plaque bacteria in association with two preparations of MUC5B mucins in solution and in biofilms. Microbiology (Reading). 2009;155:53–60. https://doi.org/10.1099/mic.0.022111-0, PMID:19118346

[21] Frenkel ES, Ribbeck K. Salivary mucins in host defense and disease prevention. J Oral Microbiol. 2015;7:29759. https://doi.org/10.3402/jom.v7.29759, PMID:26701274

[22] Kavanaugh NL, Zhang AQ, Nobile CJ, Johnson AD, Ribbeck K. Mucins suppress virulence traits of Candida albicans. MBio. 2014;5:e01911-14. https://doi.org/10.1128/mBio.01911-14, PMID:25389175

[23] Gale MS, Darvell BW. Thermal cycling procedures for laboratory testing of dental restorations. J Dent. 1999;27:89–99. https://doi.org/10.1016/S0300-5712(98)00037-2, PMID:10071465

[24] Türkcan İ, Nalbant AD, Bat E, Akca G. Examination of 2-methacryloyloxyethyl phosphorylcholine polymer coated acrylic resin denture base material: surface characteristics and *Candida albicans* adhesion. J Mater Sci Mater Med. 2018;29:107. https://doi.org/10.1007/s10856-018-6116-7, PMID:29971499

[25] Yoshijima Y, Murakami K, Kayama S, Liu D, Hirota K, Ichikawa T, *et al.* Effect of substrate surface hydrophobicity on the adherence of yeast and hyphal *Candida*. Mycoses. 2010;53:221–6. https://doi.org/10.1111/j.1439-0507.2009.01694.x, PMID:19671080

[26] Sarkar A, Xu F, Lee S. Human saliva and model saliva at bulk to adsorbed phases – similarities and differences. Adv Colloid Interface Sci. 2019;273:102034. https://doi.org/10.1016/j.cis.2019.102034, PMID:31518820