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Evaluation of the effectiveness of bioactive glass fillers against *Candida albicans adhesion* to PMMA denture base materials: An in vitro study



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KEYWORDS

Antifungal agent; Bioactive glass; Acrylic resin; Removable Dental prostheses **Abstract** *Background:* Dentures with antimicrobial properties are desirable for preventing *Candida albicans* adhesion. This study was to assess the effectiveness of bioactive glass (BAG) on *C. albicans* adhesion, surface roughness, and hardness of denture base materials.

Methods: Heat-polymerized (HP) and autopolymerized (AP) acrylic resins were used to fabricate 240 disk specimens (120/material, 60/*C. albicans*, 60/surface roughness and hardness). Specimens were divided into five groups (n = 10) based on the BAG concentration: 0.5, 1.5, 3, 5, and 7.5 wt% of the acrylic powder, with a control group comprised of unmodified specimens. Direct culture method was used to assess *C. albicans* adhesion. A profilometer and Vickers hardness test were used to measure surface roughness and hardness respectively. Analysis of variance (ANOVA) and post hoc Tukey's test were used for data analysis ($\alpha = 0.05$).

Results: BAG addition significantly decreased the *C. albicans* count when compared with the control group (P < 0.001) for both HP and AP. Regarding surface roughness, there was no change

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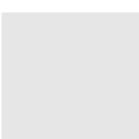
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in the HP acrylic resins (P > 0.05), while the AP acrylic resins exhibited significantly higher surface roughness with BAG addition (P < 0.001). The hardness of the HP and AP acrylic resins were significantly higher with the addition of BAG (P < 0.001).

Conclusions: The addition of BAG to HP and AP acrylic resins effectively decreases *C. albicans* adhesion. The roughness of AP acrylic resins increases with the addition of BAG, while the hardness of both HP and AP acrylic resins increase with the addition of BAG.

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1. Introduction

Denture stomatitis (DS) is considered a very common disease among denture wearers (Gendreau and Loewy, 2011). DS presents as inflammation and erythema of the denture foundation area (Kossioni, 2010). The prevalence of DS among individuals who wear removable dental prostheses ranges from 15 % to over 70 % (Gendreau and Loewy, 2011). Poor oral hygiene, poor denture fit, low saliva flow, bacterial infection, and fungal infection (primarily *Candida*) are associated with DS. However, *Candida albicans (C. albicans)* is the primary microbe responsible for DS (Vanden Abbeele et al., 2008; Webb et al, 1998).

The use of denture base materials with appropriate surface roughness and hardness is recommended for denture longevity. A key factor in the adhesion of *C. albicans* is the intaglio surface roughness (Gad and Fouda, 2020; Gleiznys et al, 2015; Zamperini et al, 2010). Furthermore, a denture base material with adequate hardness is required to ensure adequate resistance to scratching during denture cleaning (Gad and Fouda, 2020). However, this considerable surface roughness facilitates microbial attachment due to the large available surface area, leading to microbial colonization (Sterzenbach et al, 2020).

For the preservation of the remaining teeth, dental materials with ion releasing properties and bioactive ingredients (fluoride, calcium, or phosphate ions) are essential for fluoride ion release and recharge, which initiates remineralization and improves tooth resistance to demineralization (Burnett et al, 2020; Raszewski et al, 2021;Verma et al, 2015). Furthermore, the presence of these ions in oral fluids contributes to antibacterial and antifungal action (Flisfisch et al, 2008).

Oral antifungal agents have been used for the treatment of DS, e.g., nystatin and fluconazole (Gad and Fouda, 2020). Although these antifungal agents are effective in treating DS, they have some toxic side effects and promote the development of resistant strains (Gad and Fouda, 2020). For example, chlorhexidine oral mouthwash has splendid antimicrobial effects at low concentrations (0.12 %) but induces a burning sensation in the oral mucosa (Ellepola and Samaranayake, 2001; Gad and Fouda, 2020). Furthermore, using sodium hypochlorite at 0.5 % concentration for 10 min a day will expedite the elimination of denture plaque; however, it will cause strength decreasing, resin surface deterioration and discoloration (Barnabe et al, 2004; Gad and Fouda, 2020).

Because prevention of biofilm formation is critical, incorporating fillers with antimicrobial properties into polymethyl methacrylate (PMMA) denture base materials has been proposed (Gad and Fouda, 2020; Shibata et al, 2007). Apatite coated titanium dioxide fillers (5 wt%) mixed with denture base acrylic resins have an antifungal effect on C. albicans while retaining the desired mechanical properties. However, if the powder exceeds 5 wt%, the mechanical properties are adversely affected (Shibata et al, 2007). Furthermore, there is a sustained release of fluoride ions from the denture base material in constant contact with the remaining dentition, which is favorable (Raszewski et al, 2021). It has been reported that incorporating pre-reacted glass-ionomer fillers into acrylic denture base materials significantly reduces C. albicans adhesion without negatively impacting the physical properties of the resin (Tsutsumi et al, 2016). In addition, Al-Bakri et al. evaluated the effect of adding fluoridated glass fillers to PMMA denture base resin on microbial adherence and found that microbial adhesion decreases due to the release of fluoride ions (Al-Bakri et al, 2014a). In previous studies (Al-Bakri et al, 2014b; Kamijo et al, 2009), denture base resin containing bioactive materials such as fluoridated glass fillers (Al-Bakri et al, 2014b) and surface pre-reacted giomer has been proposed as a feasible system for fluoride release (Kamijo et al, 2009), and fluoride release in the surrounding mediums was reported.

Bioactive glass (BAG) 45S5 has some antimicrobial efficacy via the release of Na⁺ and Ca₂⁺ ions when suspended in an aqueous solution, creating a high pH environment, which is intolerable to microbiota (Waltimo et al, 2007). Furthermore, it has been suggested that silica released from BAG contributes to the antibacterial effect, as silica may directly inhibit bacterial viability by acting as surfactants at solid–liquid interfaces (Zehnder et al, 2006).

BAG has some antimicrobial efficacy, as proven by studies conducted in the medical field (Gonzalez Moreno et al., 2020; Waltimo et al, 2007; Zehnder et al, 2006). In a study conducted by Gonzalez Moreno et al. (2020) that found an efficient microbial growth inhibition effect with BAG. In a study was done by Waltimo et al, (2007) where they used BAG (amorphous nanoparticles, 20- to 60-nm size) to look into its antimicrobial efficacy and had showed an antimicrobial ability which helped to the continuous liberation of alkaline species. Recently, Chaichana et al. (2022) deduced the ability if ion release and inhibition of the growth of cariogenic bacteria upon the addition of BAG to orthodontic adhesive. However, the effectiveness of its antifungal properties in denture base material is yet to be investigated. Therefore, this study assessed the effectiveness of BAG at hindering C. albicans adhesion to PMMA denture base materials and investigates the correlation between this effect and the surface roughness and hardness of BAG-modified denture base materials. The research hypothesis is that BAG decreases the adhesion of C. albicans to denture base materials and improves the surface characteristics of PMMA/BAG denture base composites.

2. Materials and methods

This in vitro study was conducted at college of dentistry, Imam Abdulrahman Bin Faisal University. Ethical Approval (# 202108). In conducting this study, the sample size was calculated based on a previous study (Nawasrah et al, 2018). Hence, there were a total of 240 acrylic resin disk specimens, including 120 heat-polymerized (HP) acrylic resins and 120 autopolymerized (AP) acrylic resins. Sixty disks were used to investigate surface roughness and hardness, while another 60 disk specimens were used in the C. albicans adhesion test. The specimens were further divided into six subgroups (n = 10) based on the BAG concentration. Wax specimens were fabricated using a 10×2 mm silicon mold and then invested within a metal flask. Subsequently, all the wax was melted away and the mold was packed with acrylic resin and processed following the conventional method for denture processing (Hamid et al. 2019) (Gad et al. 2019) (AlBin-Ameer et al. 2019).

Two types of acrylic resin were selected: HP (Major Base 20, Major, Moncalieri, Italy) and AP (Major Repair, Major, Moncalieri, Italy). The BAG (Mo-sci, 45S5, 53 µm, Moncalieri, Italy) was weighed and added to acrylic powder in the following concentrations: 0.5, 1.5, 3, 5, and 7.5 wt%, using a digital balance. The mixture was then properly mixed, forming homogenous PMMA or BAG mixtures as described in the study by Raszewski et al (2021). The HP and AP acrylic resins were formulated and mixed according to the manufacturer's instructions. For the HP specimens, the mixture was packed at the doughy stage. This was followed by polymerization in a thermostat-controlled water bath: first, in cold water; then the water was heated to 70 °C for duration of 90 min: the temperature was then increased to 100 °C for 30 min; and lastly, the polymer was allowed to cool at room temperature for 1 h. For the AP specimens, the ingredients were mixed properly following the instructions of the manufacturer, packed in a metal mold, and then placed in a pressure pot for 15 min under 30 Ib/inch² pressure.

The acrylic disks were retrieved after complete polymerization and then finished with a tungsten bur. An automatic polisher (Metaserv® 250 Grinder-polisher) was used for the polishing step, polishing with a 500-grit sandpaper for 30 s under continuous water flow. The prepared specimens were evaluated, and specimens with voids, porosity, or variations in dimensions were omitted from the study sample. The selected specimens were then stored in distilled water at 37 °C for 48 h. All finishing and polishing procedures were performed by a single operator.

Before preparing the *C. albicans* assay, the acrylic disks were cleaned using an ultrasonic cleaner containing sterilized distilled water and were then subjected to ultraviolet (UV) light for 30 min (Gulati et al, 2018). Sabouraud dextrose plates (SDA) (Acumedica Co., Manufacturers, Inc.) were used to culture *C. albicans* (ATCC 10231) at a temperature of 30 °C for 48 h. A fresh single colony was inoculated overnight in sabouraud dextrose broth (SDB; Acumedica Co., Manufacturers, Inc., Lansing, MI, USA) at a temperature of 30 °C with shaking. Using a spectrophotometer, the *C. albicans* culture was standardized to 1×107 cells/ml. To allow for biofilm formation, each acrylic disk was immersed and incubated in a 200 µL standardized fungal broth for 48 h at 37 °C. After incubation, the acrylic disks were washed twice using phosphate-buffered

saline (PBS) to eliminate non-adherent cells. The specimens with adherent cells were placed within sterile tubes containing 1 mL of PBS. The adherent cells to acrylic were detached using vortex for 10 min, and then the tubes were centrifuged at 4500 rpm for 5 min (Drago et al, 2018; Gad et al, 2017a; Rao et al, 2015). The acrylic specimens were removed from the tubes, leaving behind a centrifuged solution. Subsequently, 10 mL of centrifuged solution was diluted serially, spread on a petri dish containing Blood agar (5 % of sheep blood, SPML), and incubated for 24 h at 37 °C. C. albicans colonies were counted using a direct culture method, colony-forming unit (CFU). A marker pen counter (Colony Counter; Bel-Art Scienceware, Wayne, NJ, USA) was used to count the number of C. albicans colonies in each quadrant, and the total was multiplied by the dilution factor exhibited, yielding the Candida count (CFU/mL).

A noncontact profilometer (Contour GT; Bruker Nano Inc., Tucson, AZ) was used to measure surface roughness (Ra) using a linear variable differential transformer adopted to measure the surface morphology of three different areas of a specimen and calculate the mean Ra (μ m) for each specimen.

The hardness of all specimens was measured using the Vickers hardness test (Wilson Hardness, ITW Test & Measurement GmbH, Shanghai, China), with a diamond pyramid indenter loading applied to a spot for 30 *sec* with a 50 g load at three different locations on each specimen. The average of the values from the three locations was then calculated to obtain the Vickers hardness number (VHN) for each specimen.

The Shapiro–Wilk test was used to check the normality of the data, and insignificant p-values of the test suggested that the data were normally distributed. Hence, parametric tests were used for the statistical analysis. One-way analysis of variance (ANOVA) was used to evaluate the effect of variation in the concentration levels on the tested properties, followed by the post hoc Tukey's test for pairwise comparison. P-values < 0.05 were considered statistically significant.

3. Results

Based on the one-way ANOVA results (Table 1) for the HP and AP groups, all results are statistically significant (P < 0.001), except for the Ra results for the HP groups (P = 0.257). The mean, SD, and significance of the tested properties for the HP and AP groups are summarized in Table 2. For the HP groups, in comparison to the control group, the C. albicans count was significantly lower with the addition of BAG (P < 0.001), except for the 0.5 % subgroup (P = 0.059). Comparing the BAG subgroups, the C. albicans count was significantly lower as the BAG concentrations increased from 1.5 % to 5 % (P < 0.001), while the lowest C. albicans count was recorded at 5 % (1480 \pm 385.3 CFU/ mL) (Fig. 1). For the AP groups, in comparison to the control group, C. albicans was significantly lower with BAG addition (P < 0.001), except for the 0.5 % subgroup (P = 0.741). Comparing the BAG subgroups, the C. albicans count was significantly lower between 1.5 %, 3 %, 5 %, and 7.5 % BAG (P < 0.001), with no significant difference in the C. albicans count between the 3 % 5 %, 7.5 % subgroups (P > 0.05). The lowest C. albicans count was recorded for the 5 % subgroup (7180 \pm 709.9 CFU/mL) (Fig. 2).

Resin type	Properties	Groups	Sum of Squares	df	Mean Square	F	P-value
Heat-polymerized	Candida Count	Between Groups	3015865333.3	5	603173066.667	1050.214	0.000*
	(CFU/mL)	Within Groups	31014000.0	54	574333.333		
		Total	3046879333.3	59			
	Ra	Between Groups	0.082	5	0.016	1.353	0.257
	(μ m)	Within Groups	0.651	54	0.012		
		Total	0.733	59			
	Hardness	Between Groups	375.741	5	75.148	7.730	0.000*
	(VHN)	Within Groups	524.981	54	9.722		
		Total	900.722	59			
Auto-polymerized	Candida Count	Between Groups	2694706033.3	5	538941206.667	426.465	0.000*
	(CFU/mL)	Within Groups	68242040.0	54	1263741.481		
		Total	2762948073.3	59			
	Ra	Between Groups	1.632	5	0.326	25.538	0.000*
	(µm)	Within Groups	0.690	54	0.013		
		Total	2.322	59			
	Hardness	Between Groups	395.609	5	79.122	10.306	0.000*
	(VHN)	Within Groups	414.574	54	7.677		
		Total	810.183	59			

Table 1	One-way ANOVA	results of heat-	and auto- po	lymerized a	acrylic re	sins for all	tested	properties.
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From the Ra results for the HP groups, there are no significant differences between any of the test groups (P = 0.257), with the lowest Ra recorded for the 5 % subgroup ($0.4 \pm 0.14 \mu m$) and the highest for the 1.5 % subgroup ($0.5 \pm 0.14 \mu$ m). For the AP groups, in comparison to the control group, the Ra was significantly higher with 3 %-7.5 % BAG addition (P < 0.001). The Ra of the 0.5 % subgroup was lower than that of the control group, but the difference was insignificant. In between the BAG subgroups, the Ra was consistently greater at higher BAG concentrations (0.5-7.5 %), with the lowest Ra ($0.51 \pm 0.07 \mu m$) at 0.5 % and the highest ($0.94 \pm 0.14 \mu m$) at 7.5 %.

The hardness of the HP groups was greater with the addition of BAG (P < 0.001), except for the 0.5 % subgroup (p = 0.611). Comparing the BAG subgroups, there was no significant difference (p > 0.05), with the lowest hardness value recorded at 0.5 % (41.2 ± 1.6 VHN) and the highest at

1.5 % and 3 % (47.6 \pm 2.3 VHN) and (47.6 \pm 3.2 VHN), respectively. For the AP groups, the hardness was significantly higher with the addition of BAG (P < 0.001), while there was no significant difference between all BAG subgroups (P > 0.05).

4. Discussion

Ion release and the rechargeability of denture base material containing BAG were reported in a recent study (Raszewski et al, 2021), which recommended further research on the anticipated antifungal and antibacterial action of newly proposed denture base composite. Thus, this study investigated the antifungal efficacy and surface characteristics of HP and AP denture base materials containing different concentrations of BAG. The research hypothesis that adding BAG to PMMA

Resin type	Tested Properties	BAG concentrations							
		0 % Mean ± SD	0.5 % Mean ± SD	1.5 % Mean ± SD	3 % Mean ± SD	5 % Mean ± SD	7.5 % Mean ± SD		
Heat- polymerized	Candida Count (CFU/mL)	18580 [±] 896.6 ^a	$17640^{\pm}848.7^{a}$	12780 ± 935.5	4970 ± 857.7	$1480 \pm 385.3^{\rm b}$	1930 ± 402.9^{b}		
	Ra (µm) Hardness (VHN)	$\begin{array}{rrr} 0.41 \ \pm \ 0.07 \\ 42.0 \ \pm \ 3.9 \ ^{a,b,c} \end{array}$	$\begin{array}{rrr} 0.47 \ \pm \ 0.05 \\ 41.2 \ \pm \ 1.6^{a,d,e} \end{array}$		$\begin{array}{rrr} 0.47 \ \pm \ 0.1 \\ 47.6 \ \pm \ 3.2^{f,i,j} \end{array}$	$\begin{array}{r} 0.4 \ \pm \ 0.14 \\ 44.8 \ \pm \ 3.4^{b,d,g,I,h} \end{array}$	$\begin{array}{l} 0.45 \ \pm \ 0.14 \\ 43.6 \ \pm \ 4.0^{c,e,h,j,h} \end{array}$		
Auto- polymerized	Candida Count (CFU/mL)	23340 ± 972.7 ^a	22137 ± 604.5^{a}	15016 ± 893.9	8270 ± 922.6^{b}	7180 ± 709.9^{b}	8130 ± 482.2^{b}		
	Ra (µm)	$0.53 \pm 0.67^{a,b}$	$0.51 \ \pm \ 0.07^{a,c}$	$0.6~\pm~0.14^{b,c,d}$	$0.73\ \pm\ 0.14^{d,e}$	$0.88 \pm 0.07 \ ^{\rm e,f}$	$0.94~\pm~0.14^{\rm f}$		
	Hardness (VHN)	$33.8 \pm 2.5^{a,b}$	$38.2~\pm~2.9^{\rm c,d}$	$38.6~\pm~3.0^{\rm c}$	$38.8~\pm~3.2^d$	$32.5 \pm 3.0^{a,e}$	$34.2 \pm 2.1^{b,e}$		

Table 2 Mean and SD of tested properties of heat- and auto-polymerized acrylic resins according to bioactive glass filler concentrations.

Same alphabets in each row showed statistically insignificant difference.

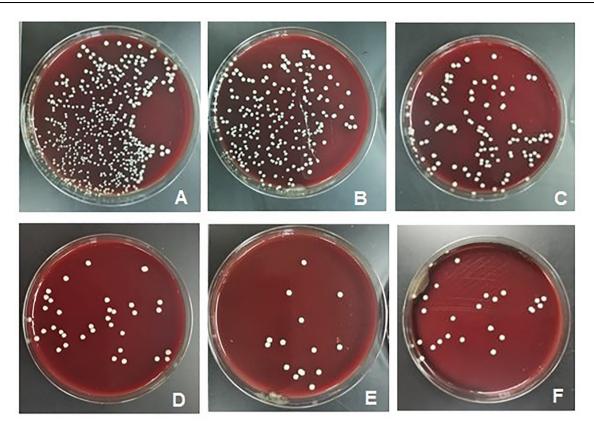


Fig. 1 A-F: Direct culture method for Candida count of HP groups. A) Unmodified, B) 0.5 wt% BAG, C) 1.5 wt% BAG, D) 3 wt% BAG, E) 5 wt% BAG, and F) 7.5 wt% BAG.

denture base materials imbues antifungal efficacy and improves the surface characteristics was partially confirmed. All tested properties returned either significantly different values than those of the control group, except for the Ra of the HP acrylic resin specimens, which exhibited no alterations at different BAG concentrations.

The results show significant differences in both the HP and AP PMMA with the addition of BAG. In a couple of similar studies, it was reported that the addition of BAG imbues antimicrobial properties (Al-Bakri et al, 2014a; Drago et al, 2018; Rao et al, 2015; Waltimo et al, 2007). The decreased C. albicans count may be attributed to the ions released from the BAG and the alteration of the pH environment alterations, which inhibit cell viability (Sepulveda et al, 2001; Waltimo et al, 2007; Zehnder et al, 2006). The ion release may be due to the dissolution of the inorganic contents of the BAG filler (Itota et al, 2005). This pattern of ions release has been described in previous studies in which leachable glass fillers dispersed in a polymer matrix were water soluble and capable of releasing ions into the storage medium (Al-Bakri et al, 2014b). With particle incorporation in denture base resins, some particles are distributed throughout the resin matrix, while others are distributed across the surface of the specimens. The imbued antifungal efficacy may be explained by the presence of these particles on the surfaces of the specimens. In addition to the leaching out of ions in surrounding fluids with sustained release action giving long-term effect of this composite material (Sepulveda et al, 2001).

In terms of BAG concentration, the *C. albicans* count decreased as the BAG concentration increased to 5 %. This is in agreement with a previous study (Rao et al, 2015). which reports that antimicrobial efficacy against *S. salivarius* increases as BAG concentration increases.

The leaching out of particles extracts residual monomers, leaving voids on the surface and within the polymer matrix. These voids induce recharge solutions to diffuse deeper into the polymer matrix, which leads to greater potential for the release and storage of ions (Xu and Burgess, 2003). Moreover, the key factor in the absorption of solutions is the hydrophilic nature of PMMA denture base materials, and this factor increases ions uptake and release from dissolved fluoridated glass fillers. However, further studies are needed for a complete understanding of the mechanism of ions release from denture base materials containing BAG fillers (Al-Bakri et al, 2014b).

There is a variation in the roughness values for the HP and AP test groups due to material properties and polymerization methods. This is confirmed by the larger variations in the AP values than in the HP values. This explains the colony count for the AP specimens being greater than that for the HP specimens at each BAG concentration (Fig. 1 and Fig. 2). The insignificant increase in Ra in HP specimens and the significant increase in Ra in AP specimens may be due to the polymerization technique: in the auto-polymerization technique, the BAG interfered with the initiator and retarded the polymerization reaction. The increased residual monomer levels and some soluble particles leach out with the monomer, leaving void areas

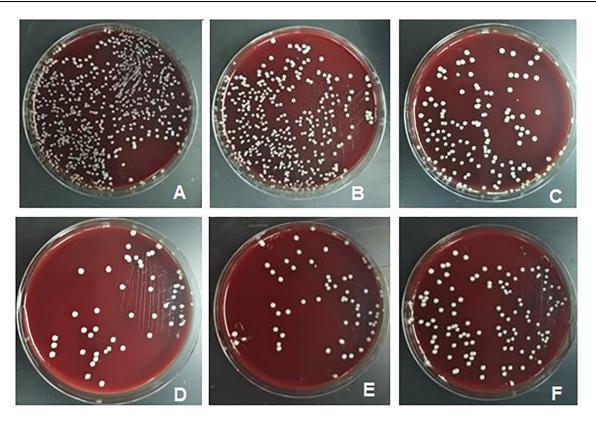


Fig. 2 A-F: Direct culture method for Candida count of AP groups. A) Unmodified, B) 0.5 wt% BAG, C) 1.5 wt% BAG, D) 3 wt% BAG, E) 5 wt% BAG, and F) 7.5 wt% BAG.

responsible for changes in Ra (Attar and Onen, 2002) which confirmed as the concentration increase the Ra increased.

Hardness was higher with the addition of BAG at different concentrations for both the HP and AP specimens. The presence of BAG on the surface of the denture materials may be the primary reason for the increased hardness. In agreement with a previous study (Gad et al., 2017b), which reported that the presence of silica particles increased the hardness of denture base material, and this increase is concentration dependent. Comparing the hardness values obtained for the HP and AP specimens, the lower values for the AP specimens were due to the presence of residual monomers, which act as a plasticizer and induce poor mechanical properties and low scratch resistance (Gad et al., 2017b; Xu and Burgess, 2003). Elboraey et al, (2020) found a decrease in hardness with the addition of hydroxyapatite nanoparticles (HA-NP), which does not agree with the finding of the present study. The different results may be due to differences in filler type, size, and concentrations used in these studies.

In terms of the effect of concentration, the highest hardness values were obtained at 1.5 % and 3 % in the HP specimens and 0.5 %, 1.5 %, and 3 % in the AP specimens. In denture base resins, a low concentration of the filler load may result in a homogenous batter mix by filling the voids in the interpolymeric chains and limiting the movement of the chains (Aldegheishem et al, 2021). Similar findings have been reported in the literature regarding the addition of silica-based fillers to PMMA increasing hardness (Gad et al., 2017b). All these findings confirm that BAG-modified denture base resins exhibit a

significant improvement in hardness compared to unmodified resins.

There is a correlation between C. albicans adhesion and the surface roughness of dentures, as the roughness increases, Candida adhesion increases. The Ra of HP showed no changes while increase with AP; however, the decrease in Candida count was concentration dependent, regardless of resin type and roughness. Although there was an insignificant increase in the roughness of HP specimens and a significant increase in the roughness of AP specimens at some concentrations, the antifungal behavior was the same. This finding may be attributed to the anticipated antifungal properties of BAG primarily due to the inherent antimicrobial effect of its composition irrespective of the surface characteristics. Furthermore, the observation that increased roughness increased C. albicans adhesion is evidence of the antifungal properties of BAG, even at high Ra values. Moreover, with greater hardness, it may be surmised that dentures containing BAG exhibit considerably high resistance during mechanical brushing or when chewing hard foods. Less scratches translate to the denture remaining smooth for a long time and contributes to the prevention of Candia adhesion.

In clinical practice, dentures with antifungal properties are recommended for medically, mentally, or physically disabled patients, as well as patients who do not adhere to postoperative denture care instructions. Furthermore, long temporization is required in cases of implant treatment, and an autopolymerized denture base for temporization with antifungal properties can contribute to treatment success as it lowers the predisposing risk factors and prevents *C. albican* adhesions to denture base resins. In addition, patients using removable partial dentures with remaining teeth are liable to develop caries, and BAG-modified denture base materials could be recommended for teeth preservation in such cases.

The results of this study should be interpreted with caution because of its limitations: the specimens were not tested in the oral environment, which contains multiple types of microorganisms, salivary fluids, and enzymes that could impact the antifungal effectiveness of BAG; and aging procedures were not performed. In addition to mixing protocol limitation, in term the absence of auto-mixer. Therefore, further research on the antifungal properties of BAG in conditions that simulate the oral environment and thermal cycling effects is recommended. It is essential that any modifications made to materials to be used in removable partial dentures either maintain or improve the mechanical properties of the material.

5. Conclusion

The addition of BAG to HP and AP acrylic resins decreases *C. albicans* adhesion. No differences were observed in the surface roughness of HP acrylic resins with the addition of BAG, while the roughness of AP acrylic resins increased. The hardness of both the HP and AP acrylic resins improved with the incorporation of BAG. In clinical practice, the addition of BAG at concentrations of 1.5 %, 3 %, and 5 % is a potentially effective method for decreasing *C. albicans* adhesion and for use in the prevention and treatment of DS.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- Al-Bakri, I.A., Harty, D., Al-Omari, W.M., Swain, M.V., Chrzanowski, W., Ellakwa, A., 2014a. Surface characteristics and microbial adherence ability of modified polymethylmethacrylate by fluoridated glass fillers. Aust. Dent. J. 59 (4), 482–489.
- Al-Bakri, I.A., Swain, M.V., Naoum, S.J., Al-Omari, W.M., Martin, E., Ellakwa, A., 2014b. Fluoride release, recharge and flexural properties of polymethylmethacrylate containing fluoridated glass fillers. Aust. Dent. J. 59 (2), 208–214.
- AlBin-Ameer, M.A., Alsrheed, M.Y., Aldukhi, I.A., Matin, A., Khan, S.Q., Abualsaud, R., et al, 2019. Effect of protective coating on surface properties and candida albicans adhesion to denture base materials. J Prosthodont. 29 (1), 80–86.
- Aldegheishem, A., AlDeeb, M., Al-Ahdal, K., Helmi, M., Alsagob, E. I., 2021. Influence of reinforcing agents on the mechanical properties of Denture Base Resin: A systematic review. Polymers. 13 (18), 3083.
- Attar, N., Onen, A., 2002. Fluoride release and uptake characteristics of aesthetic restorative materials. J. Oral Rehabil. 29, 791–798.
- Barnabe, W., Mendonca Neto, T., Pimenta, F.C., Pegoraro, L.F., Scolaro, J.M., 2004. Efficacy of sodium hypochlorite and coconut soap used as disinfecting agents in the reduction of denture stomatitis, streptococcus mutans and candida albicans. J. Oral Rehabil. 31 (5), 453–459. https://doi.org/10.1111/j.1365-2842.2004.01254.x.

- Burnett, G., Nehme, M., Parkinson, C., Karwal, R., Badrock, T., Thomas, G.V., et al, 2020. A randomised oral fluoride retention study comparing intra-oral kinetics of fluoride-containing dentifrices before and after dietary acid exposure. Arch. Oral Biol. 119, 104891.
- Chaichana, W., Insee, K., Chanachai, S., Benjakul, S., Aupaphong, V., Naruphontjirakul, P., Panpisut, P., 2022 Apr 22. Physical/mechanical and antibacterial properties of orthodontic adhesives containing Sr-bioactive glass nanoparticles, calcium phosphate, and andrographolide. Sci. Rep. 12 (1), 6635. https://doi.org/10.1038/ s41598-022-10654-6.
- Drago, L., Toscano, M., Bottagisio, M., 2018. Recent evidence on bioactive glass antimicrobial and antibiofilm activity: A minireview. Materials. 11 (2), 326.
- Elboraey, A.N., Abo-Almaged, H.H., El-Ashmawy, A.A., Abdou, A. R., Moussa, A.R., Emara, L.H., et al, 2020. Biological and mechanical properties of denture base material as a vehicle for novel hydroxyapatite nanoparticles loaded with drug. Adv Pharm Bull. 11 (1), 86–95.
- Ellepola, A.N.B., Samaranayake, L.P., 2001. Adjunctive use of chlorhexidine in oral Candidoses: A Review. Oral Dis. 7, 11–17.
- Flisfisch, S., Meyer, J., Meurman, J.H., Waltimo, T., 2008. Effects of fluorides on candida albicans. Oral Dis. 14 (4), 296–301.
- Gad, M.M., Al-Thobity, A.M., Rahoma, A., Abualsaud, R., Al-Harbi, F.A., Akhtar, S., 2019. Reinforcement of PMMA denture base material with a mixture of ZrO₂ nanoparticles and glass fibers. Int J Dent. 2019, 1–11. https://doi.org/10.1155/2019/2489393. PMID: 30809260; PMCID: PMC6369466.
- Gad, M., Al-Thobity, A.M., Shahin, S., Alsaqer, B., Ali, A., 2017a. Inhibitory effect of zirconium oxide nanoparticles on Candida albicans adhesion to repaired polymethyl methacrylate denture bases and interim removable prostheses: A new approach for denture stomatitis prevention. Int. J. Nanomed. 12, 5409–5419. https://doi.org/10.2147/IJN.S142857.
- Gad, M., Fouda, S., Al-Harbi, F., Näpänkangas, R., Raustia, A., 2017b. PMMA Denture Base Material Enhancement: A review of fiber, filler, and nanofiller addition. Int J Nanomedicine. 12, 3801– 3812.
- Gad, M., Fouda, S., 2020. Current Perspectives and the future of candida albicans-associated denture stomatitis treatment. Dent Med Probl. 57, 95–102. https://doi.org/10.17219/dmp/112861.
- Gendreau, L., Loewy, Z.G., 2011. Epidemiology and etiology of denture stomatitis. J Prosthodont. 20, 251–260. https://doi.org/ 10.1111/j.1532-849X.2011.00698.x.
- Gleiznys, A., Zdanavičienė, E., Žilinskas, J., 2015. Candida albicans importance to denture wearers. A literature review. Stomatologija. 17, 54–66.
- Gonzalez Moreno, M., Butini, M.E., Maiolo, E.M., Sessa, L., Trampuz, A., 2020. Antimicrobial activity of bioactive glass S53P4 against representative microorganisms causing osteomyelitis

 real-time assessment by Isothermal microcalorimetry. Colloids Surf., B 189, 110853.
- Gulati, M., Arevalo, A., Bapat, P., Perry, A.M., Gonzalez, R.E., Ennis, C.L., et al, 2018. In Vitro Culturing and Screening of Candida Albicans Biofilms. Curr Protoc Microbiol. 50 (1), e60.
- Hamid, S.K., Al-Dubayan, A.A., Al-Awami, H., Khan, S.Q., Gad, M. M., 2019. In vitro assessment of the antifungal effects of neem powder added to polymethyl methacrylate denture base material. J. Clin. Exp. Dent. 11, 170–178. https://doi.org/10.4317/jced.55458. eCollection 2019 Feb.
- Itota, T., Al-Naimi, O.T., Carrick, T.E., Yoshiyama, M., McCabe, J. F., 2005. Fluoride release from aged resin composites containing fluoridated glass filler. Dent. Mater. 21 (11), 1033–1038.
- Kamijo, K., Mukai, Y., Tominaga, T., Iwaya, I., Fujino, F., Hirata, Y., et al, 2009. Fluoride release and recharge characteristics of denture base resins containing surface pre-reacted glass-ionomer filler. Dent. Mater. J. 28 (2), 227–233.

- Kossioni, A.E., 2010. The prevalence of denture stomatitis and its predisposing conditions in an older Greek population. Gerodon-tology. 28, 85–90. https://doi.org/10.1111/j.1741-2358.2009.00359.
 x. Epub 2010 Jan 14.
- Nawasrah, A., Gad, M.M., Zayat, M.E., 2018. Effect of henna addition on the surface roughness and hardness of polymethylmethacrylate denture base material: An in vitro study. J. Contemp. Dent. Pract. 19 (6), 732–738.
- Rao, S.R., Chandrasekar, R.S., Lavu, V., Kumar, K., 2015. Evaluation of antimicrobial properties of bioactive glass used in regenerative periodontal therapy. J Indian Soc Periodontol. 19 (5), 516.
- Raszewski, Z., Nowakowska, D., Wieckiewicz, W., Nowakowska-Toporowska, A., 2021. Release and recharge of fluoride ions from acrylic resin modified with bioactive glass. Polymers. 13 (7), 1054.
- Sepulveda, P., Jones, J.R., Hench, L.L., 2001. Characterization of melt-derived 45S5 and sol-gel-derived 58s bioactive glasses. J. Biomed. Mater. Res. 58, 734–740. https://doi.org/10.1002/ jbm.10026.
- Shibata, T., Hamada, N., Kimoto, K., Sawada, T., Sawada, T., Kumada, H., et al, 2007. Antifungal effect of acrylic resin containing apatite-coated TiO₂ photocatalyst. Dent. Mater. J. 26, 437–444. https://doi.org/10.4012/dmj.26.437. PMID: 17694755.
- Sterzenbach, T., Helbig, R., Hannig, C., Hannig, M., 2020. Bioadhesion in the oral cavity and approaches for biofilm management by surface modifications. Clin Oral Investig. 24 (12), 4237–4260.
- Tsutsumi, C., Takakuda, K., Wakabayashi, N., 2016. Reduction of candida biofilm adhesion by incorporation of prereacted glass ionomer filler in denture base resin. J. Dent. 44, 37–43. https://doi. org/10.1016/j.jdent.2015.11.010. Epub 2015 Dec 2.

- Vanden Abbeele, A., de Meel, H., Ahariz, M., Perraudin, J.-P., Beyer, I., Courtois, P., 2008. Denture contamination by yeasts in the elderly. Gerodontology. 25, 222–228. https://doi.org/10.1111/ j.1741-2358.2007.00247.x. Epub 2008 Jul 26.
- Verma, A., Khurshid, S., Parveen, F., Khanna, S., Pandey, P., 2015. Remineralization : An approach towards conservation of Tooth. Journal of Evolution of Medical and Dental Sciences. 4 (61), 10713–10719.
- Waltimo, T., Brunner, T.J., Vollenweider, M., Stark, W.J., Zehnder, M., 2007. Antimicrobial effect of nanometric bioactive glass 45s5. J. Dent. Res. 86, 754–757.
- Webb, B.C., Thomas, C.J., Willcox, M.D., Harty, D.W., Knox, K.W., 1998. Candida-associated denture stomatitis. Aetiology and management: A review: PART1. factors influencing distribution of candida species in the oral cavity. Aust. Dent. J. 43 (1), 45–50. https://doi.org/10.1111/j.1834-7819.1998.tb00152.x.
- Xu, X., Burgess, J.O., 2003. Compressive Strength, fluoride release and recharge of fluoride-releasing materials. Biomaterials 24 (14), 2451– 2461.
- Zamperini, C.A., Machado, A.L., Vergani, C.E., Pavarina, A.C., Giampaolo, E.T., da Cruz, N.C., 2010. Adherence in vitro of candida albicans to plasma treated acrylic resin. Effect of plasma parameters, surface roughness and salivary pellicle. Arch. Oral Biol. 55, 763–770. https://doi.org/10.1016/j. archoralbio.2010.06.015.
- Zehnder, M., Waltimo, T., Sener, B., Söderling, E., 2006. Dentin enhances the effectiveness of bioactive glass S53P4 against a strain of enterococcus faecalis. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 101 (4), 530–535. https://doi.org/10.1016/j.tripleo.2005.03.014. Epub 2005 Oct 14 PMID: 16545719.