Zinc-modified phosphate-based glass micro-filler improves Candida albicans resistance of auto-polymerized acrylic resin without altering mechanical performance

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Article

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Abstract

Colonization of auto-polymerized acrylic resin by pathogenic *Candida albicans* is a common problem for denture users. In this study, zinc-modified phosphate-based glass was introduced into an auto-polymerized acrylic resin at concentrations of 3, 5, and 7 wt.%. The mechanical properties (flexural strength, elastic modulus, microhardness, and contact angle) and surface morphology of the resultant material were investigated, and the antimicrobial effect on *C. albicans* was examined. The mechanical properties slightly differed between the control and zinc-modified phosphate-based glass samples ($p > 0.05$); however, the number of *C. albicans* colony-forming units was significantly lower in the control ($p < 0.05$). Scanning electron microscopy revealed that *C. albicans* tended not to adhere to the material. Thus, the novel materials retained the advantageous mechanical properties of unaltered acrylic resins while simultaneously exhibiting a strong antimicrobial effect in vitro.

Introduction

The rapid growth of the elderly population has resulted in a corresponding increase in the number of denture users[1]. The bases of dentures are often made of acrylic resin, especially poly(methyl methacrylate) acrylic (PMMA)[2], an easy-to-use, moldable, and affordable material that offers satisfactory aesthetics and excellent biocompatibility. However, PMMA and other denture-base resins are susceptible to microbial colonization in the oral environment[3].

The absence of ionic charges in the auto-polymerized acrylic resin prevents protective saliva molecules from adhering to the surface of the denture and thus promotes the formation of biological membranes[4]. Hydrophobic interactions and mechanical attachment (in conjunction with local roughness, surface porosity, and poor hygiene) may also induce bacterial attachment. The denture base functions as a substrate for microbial adhesion and biofilm formation, thereby resulting in denture stomatitis. This may induce additional complications such as fungal infections, which are highly significant for elderly and immunosuppressed patients[5,6].

Candida albicans is the primary pathogen in denture stomatitis, a widely recurring disease that affects approximately 11–67% of denture users[7,8]. *C. albicans* on the surface of acrylic resin used as a denture-base material or oral epithelium is usually in a non-pathogenic state; however, it induces diseases in patients with weakened immunity as well as opportunistic infections[9]. The prevention or suppression of denture stomatitis is essential because the formation of *C. albicans* biofilms is associated with severe local and systemic infections in denture users[10]. *C. albicans* is released into saliva and may be subsequently aspirated into the lower respiratory tract, inducing pneumonia in the elderly[11]. Therefore, a method to optimize the antimicrobial properties of auto-polymerized acrylic resin is necessary.

A 1–2% solution of chlorhexidine gluconate is used for the treatment of *C. albicans*-induced denture stomatitis; however, it induces discoloration[12]. Conventional methods of treating oral candidiasis involving the use of dentures are generally effective for the removal of accumulated plaque but pose

challenges for the elderly, especially those with disabilities or who require nursing care[13]. Antimicrobial agents are also effective in the control of dentures but induce toxic side effects and the development of resistant strains. This results in deterioration of the physical and mechanical properties of the dentures[3,14]. Therefore, research on inorganic metals that are effective in preventing fungal infections is being actively conducted[15,16].

Recently, certain inorganic materials have been shown to exhibit broad-spectrum biocidal effects[17,18]. Various inorganic fillers have therefore been proposed for modifying the properties of acrylic-based resin[17,19]. Most fillers are intended to augment microbial resistance[20]. To eliminate *C. albicans*, both micro-fillers and nano-fillers have been proposed[21–23]. Among these inorganic materials, zinc ions, in particular, have been explored as an alternative to conventional antimicrobials in dental materials[24,25]. They seem to offer at least some microbial resistance[21,22] but alter mechanical properties[23]: increasing the additive percentage further improves the anti-candidal response at the expense of mechanical and physical properties[26,27].

Most micro-particles change the mechanical properties of acrylic resin to which they are added because they are incompatible with its matrix structure[6]. Similar undesired changes in mechanical properties have also been observed with the use of nanoparticles, mainly due to particle agglomeration[28]. Although pre-salination is believed to improve homogeneity, a matrix-compatible micro-filler to improve the properties of acrylic-based denture resins has not been found[26,29]. Phosphate-based glass (PBG) exhibits composition-dependent chemical durability, which limits its application in aqueous solution[30–33]. By contrast, the chemical durability of zinc-modified PBG (Zn-PBG) micro-filler depends only on the glass composition[24].

From these considerations, we propose the use of Zn-PBG microparticles synthesized by zinc modification of the phosphate-dominant bioglass. We examine the key mechanical properties of the acrylic resin, evaluate the ionic elution, and comprehensively evaluate the material's resistance to *C. albicans.*

Materials And Methods

Glass preparation. To obtain glass powder, P_2O_5 (42 mol%), CaO (25.2 mol%), Na_2O (16.8 mol%), and ZnO (16 mol%) powders were mixed in a tubular shaker–mixer. The batch of mixed powders was melted in an alumina crucible at 1100°C for 1 h using an electric furnace. Subsequently, the melted glass was quenched at room temperature to obtain glass cullet. This was followed by grinding in an alumina mortar and subsequent pulverization under dry conditions using a planetary mono-mill (Pulverisette-7; Fritsch, Idar-Oberstein, Germany).

Incorporation of Zn-PBG into auto-polymerized acrylic resin. A commercially available orthodontic acrylic resin (Ortho-Jet, Lang Dental Manufacturing Co. Inc.) was used according to the manufacturer's

instructions. The material was an auto-polymerized resin system. The Zn-PBG powder was homogeneously mixed with the acrylic-resin powder at various weight concentrations (3, 5, and 7 wt.%); acrylic-resin powder without Zn-PBG was used as a control. The compositions of the control and experimental groups used in the present experiment are listed in Table 1. All the specimens were manufactured using powder and liquid mixed in a mass ratio of 3:2 and subjected to low-temperature polymerization (60°C, 4.0 bar, 15 min, Air Press Unit, Sejong Dental). Thereafter, all the specimens were polished using abrasive 800–2000 grit papers.

Table 1
Compositions of control and experimental groups in this study

Group	Group code	Auto-polymerized acrylic resin (wt.%)	Zn-PBG (wt.%)
1	Control	100	0
2	3 wt.% Zn-PBG	97	3
3	5 wt.% Zn-PBG	95	5
4	7 wt.% Zn-PBG	93	7

Determination of elemental composition of Zn-PBG powder. Field-emission scanning electron microscopy (FE-SEM; Merin, Carl Zeiss, Oberkochen, Germany) in conjunction with energy-dispersive X-ray spectroscopy (EDS) was performed to determine the elemental composition.

Flexural strength and elastic modulus. The flexural strength and elastic modulus were tested according to the International Standard ISO 20795-1. Seven specimens were manufactured for each PMMA group with dimensions of 64 × 10 × 3.3 mm^3. The prepared specimens were immersed in 10 mL of distilled water and stored at 37°C for 24 h. Thereafter, all the specimens were loaded to fracture using a universal testing machine (Model 5942, Instron, Norwood, MA, USA) with a span length of 50 mm and a crosshead speed of 5 mm/min. The flexural strength σ and elastic modulus E were calculated from

$$\sigma = \frac{3Fl}{2bh^2},$$

$$\mathrm{E} = \frac{Pl^3}{4bh^3d},$$

where F is the maximum load, l is the distance between the supports (mm), b and h are the width and height of the specimens (mm) prior to water storage, P is the load at a point in the straight-line portion of the load/displacement curve, and d is the deflection at load P (mm).

Microhardness. Three specimens (diameter, 10 mm; thickness, 2 mm) were prepared for each group. A total of 12 specimens were assessed via Vickers hardness testing (MMT-X, Matsuzawa Seiki Co., Tokyo, Japan), using a Vickers diamond indenter with load and dwell times of 50 g and 10 s, respectively. Three

points were measured randomly for each specimen, and the mean value and standard deviation were calculated.

Contact angle. The wettability of each specimen was determined via contact-angle analysis (SmartDrop, Femtobiomed Inc., Gyeonggi-do, Korea). Three samples from each group were considered (diameter, 10 mm; thickness, 2 mm). Distilled water (5 µL) was randomly dropped on the surface, and the contact angle was measured after 10 s of contact. This process was repeated three times.

Scanning electron micrographs of sample surface. Prior to measurement, all the manufactured specimens were sputter-coated using platinum to facilitate observation of the material surface. The SEM (Merin, Carl Zeiss, Oberkochen, Germany) images were obtained under an accelerating voltage of 15 kV and magnification 500×.

Standard preparation of fungal specimens. *C. albicans* ATCC 10231 was cultured at 37°C in a yeast mold (YM, Becton Dickinson and Co., Franklin Lakes, NJ, USA) medium for 24 h. A discoid specimen was prepared; thereafter, 1 mL of fungal suspension (1 x 10^8 cells/mL) was placed on each disk in a 24-well plate and incubated at 37°C for 24 h under a 95% or higher humidified atmosphere. The specimens were gently washed twice using PBS to remove any mold after incubation.

Morphology. *C. albicans* specimens prepared in the standard way were placed in 2% paraformaldehyde–glutaraldehyde in 0.1M PBS for at least 30 min at room temperature. The specimens were fixed with 1% OsO_4, which was dissolved in 0.1 M PBS for 2 h. Subsequently, they were dehydrated in ethanol, treated with isoamyl acetate, and subjected to critical-point drying (LEICA EM CPD300; Leuca, Wien, Austria). Thereafter, the specimen were subjected to Pt-ion coating (5 nm; ACE600; Leica). This was followed by examination and imaging via SEM (Merin, Carl Zeiss, Oberkoche, Germany) at 2 kV.

Colony-forming units (CFUs). *C. albicans* specimens were prepared in the standard way. The attached fungi were harvested for 5 min in 1 mL of YM via sonication (SH-2100; Saehan Ultrasonic, Seoul, Korea). The procedure was adapted from a previous study[24,34]. Subsequently, 100 µL of this fungal suspension was spread on YM agar plates and incubated at 37°C for 24 h. Thereafter, the total number of colonies was calculated.

Ion Release. Disk-shaped samples were formed using a Teflon mold with a diameter and thickness of 10 and 2 mm, respectively. Five disks were fabricated from each group, and all the samples were stored in 5 mL of distilled water containing 15 mL conical tubes. The specimens were then stored at 37°C for 24 h. The Ca, P, and Zn ions released from the specimens were detected using inductively coupled plasma-optical emission spectrometry (ICP-OES, Optima 8300, PerkinElmer, Waltham, MA, USA).

Statistical analysis. All statistical analyses were performed using IBS SPSS version 25.0 (IBM Korea Inc., Seoul, Korea) software. The level of significance was assessed at $p < 0.05$. The results of all groups were analyzed using a one-way analysis of variance followed by Tukey's post hoc test.

Results

Analysis of elemental composition of Zn-PBG powder. Figure 1 shows the amounts of C, O, Na, P, Ca, Zn, and Al in the Zn-PBG powder. The image in Fig. 1 indicates that the distribution of elements in the powder was uniform.

Flexural strength and elastic modulus. The measured flexural strength and elastic modulus are presented in Figs. 2A and 2B, respectively. The flexural strength increased with the amount of Zn-PBG, and all the groups fulfilled the 60 MPa requirements of the ISO 20795-1 standard. Although the flexural strengths of the control group (81.77 ± 6.55 Mpa), 3% Zn-PBG (72.27 ± 8.76 Mpa), and 5% Zn-PBG (72.77 ± 5.79 Mpa) showed no significant differences ($p > 0.05$), the flexural strength of the 7% Zn-PBG (69.57 ± 6.40) group differed significantly from that of the control ($p < 0.05$). The elastic modulus of all the groups showed no significant difference with increasing Zn-PBG ($p > 0.05$).

Microhardness. The microhardness results of the control and experimental groups are shown in Fig. 2C. There were no significant differences in the results across all the groups ($p > 0.05$). This indicated that the addition of Zn-PBG to PMMA did not have any effect on the surface microhardness.

Contact angle. The measured contact angles for all the groups are shown in Fig. 2D. The 5% Zn-PBG group exhibited the highest contact angle (84.94 ± 3.73). However, there were no significant differences in the results for the control and 3% Zn-PBG groups ($p > 0.05$). The 7% Zn-PBG group exhibited the lowest contact angle (73 ± 4.73); this was significantly different from the contact angle of the 5% Zn-PBG group ($p < 0.05$).

SEM images of sample surface. The SEM images of the auto-polymerized acrylic resin specimen were recorded for surface analysis (Fig. 3). There were no significant differences in the surface morphologies of each group. These results indicate that Zn-PBG did not affect the PMMA surface.

Antifungal properties. The SEM images demonstrated that the quantity of *C. albicans* adhering to the surface was lower for the Zn-PBG groups than for the control groups (Fig. 4) and decreased with an increase in the content of Zn-PBG. Furthermore, in the quantitative analysis, log (CFUs/mL) for the control was significantly higher than that for the other Zn-PBG groups ($p < 0.05$) (Fig. 5).

Ion Release. The release of Ca, P, and Na ions from each specimen is shown in Fig. 6. The results indicate an increase in the release of Ca and P ions with an increase in the amount of Zn-PBG. The release of Zn ions was not observed in the control group, in contrast to the experimental groups. The maximum release of Zn ions was from the 7% Zn-PBG group, with a significant difference compared to the control group ($p < 0.05$). The concentrations of the Ca and P ions for 3% Zn-PBG were significantly different from those for 5% and 7% Zn-PBG ($p < 0.05$).

Discussion

Auto-polymerized acrylic resin has a long history of use and is one of the most developed and efficient dental materials. However, it has no antimicrobial activity. The fabrication of an auto-polymerized acrylic resin with antimicrobial properties would be of great clinical benefit, provided that its physical and mechanical properties were unaltered. The present study has achieved this.

Initially, the effectiveness of the synthesis of Zn-PBG was demonstrated by ion mapping. The specimens were prepared via the addition of auto-polymerized acrylic resin, and their physical and mechanical properties were evaluated. Flexural strength is an important property of dentures. There was no statistically significant difference in the flexural strengths of 3% and 5% Zn-PBG as compared to that of the control. The modulus of elasticity was also largely unchanged. As the denture surface can be scratched and fractured under masticatory pressure, hardness is an important property[35]. The surface hardness of the auto-polymerized acrylic resin is also used to measure the resistance to the force applied during mastication; if the surface hardness is lowered, the stress distribution due to the masticatory force is not uniform[36]. There were no significant differences in the hardness of the experimental specimens as compared to that of the control. The contact angle of the surface affects the adhesion of bacteria: there were no significant differences in the contact angles of the experimental specimens as compared to that of the control. SEM observations likewise revealed no significant changes in morphology.

The physical strength, color tone, and surface shape of the denture-base resin are affected by the use of denture cleaners or mechanical denture management. When the denture base is worn, scratches induced by wear may roughen the surface and induce the accumulation of plaque and tartar[36]. Previous studies revealed that the presence of silver nanoparticles in resin resulted in high antifungal activity; however, deterioration such as substantial changes in material properties or color was not alleviated[29,37]. By contrast, the auto-polymerized acrylic resin containing Zn-PBG does not cause physical and mechanical differences from the control group. We argue that this was because Zn-PBG microparticles were synthesized by zinc modification of the phosphate-dominant bioglass[24].

The number of *C. albicans* colonies attached to the surface of the control was very high, as confirmed by the SEM images; however, it gradually decreased as the content of Zn-PBG increased. The quantity of *C. albicans* attached to the surface was significantly lower in the 7% Zn-PBG group than in the control group. To confirm this result quantitatively, CFU counts were measured. The results were similar to the SEM results: the control group presented a high number, and the number decreased as the Zn-PBG content increased. In particular, the 7% Zn-PBG group had less than half the CFU count of the control group.

An analysis of the ion release results indicated that the contents of Ca, P, and Zn ions were significantly higher in the experimental groups than in the control group. Increasing the Zn-ion content was correlated with a decreasing the number of *C. albicans* isolates. Zn is an important mineral that plays a role in microbial inhibition[24]. Furthermore, the antibacterial activity of Zn ions and the binding action of Ca, P, Na, and Si ions stimulate the assimilation reaction[38]. These constructive effects depend on the concentration of Zn ions released; in previous studies, they were prominent at low concentrations and

were exponentially suppressed as the zinc oxide content of the glass increased[28]. This agrees with the results of this study, in which the PMMA containing Zn-PBG exhibited an antimicrobial effect against *C. albicans*.

Our results can be utilized as basic data for the development of auto-polymerized acrylic resin with antimicrobial effects in the future. However, long-term experimental studies are required to evaluate their clinical use. Cytotoxic tests and long-term studies of the durability of the antimicrobial effect must be performed, and the findings presented here should be confirmed by experiments conducted in vivo.

Conclusion

In this study, an auto-polymerized acrylic resin containing Zn-PBG was fabricated, its physical and mechanical properties were measured, and its antimicrobial activity was confirmed. The following are the major conclusions:

1. The addition of Zn-PBG did not result in loss of flexural strength, modulus of elasticity, hardness, or contact angle.

2. *C. albicans* colony formation decreased as Zn-PBG content increased.

3. The number of Ca, P, and Zn ions released increased as the Zn-PBG content increased.

Thus, the incorporation of Zn-PBG into the experimental auto-polymerized acrylic resin inhibited colonization by *C. albicans* without jeopardizing the overall physical and mechanical properties of the auto-polymerized acrylic resin.

Declarations

Data Availability

The data will be shared at a reasonable request to the corresponding author.

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Author Contribution Statement

M.J.L, M.J.K., U.M., J.Y.S., S.H.C., and J.S.K. conceived and designed the experiments. M.J.L., M.J.K., and J.Y.S. performed all the experiments. M.J.K. and U.M. interpreted and analyzed the data. M.J.L and M.J.K. conceived the study and wrote the manuscript. S.H.C. and J.S.K. provided manuscript writing assistance

and critically revised the manuscript, adding important intellectual content. All authors reviewed and approved the final manuscript.

Competing Interests

The authors declare no conflict of interest.

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Figures

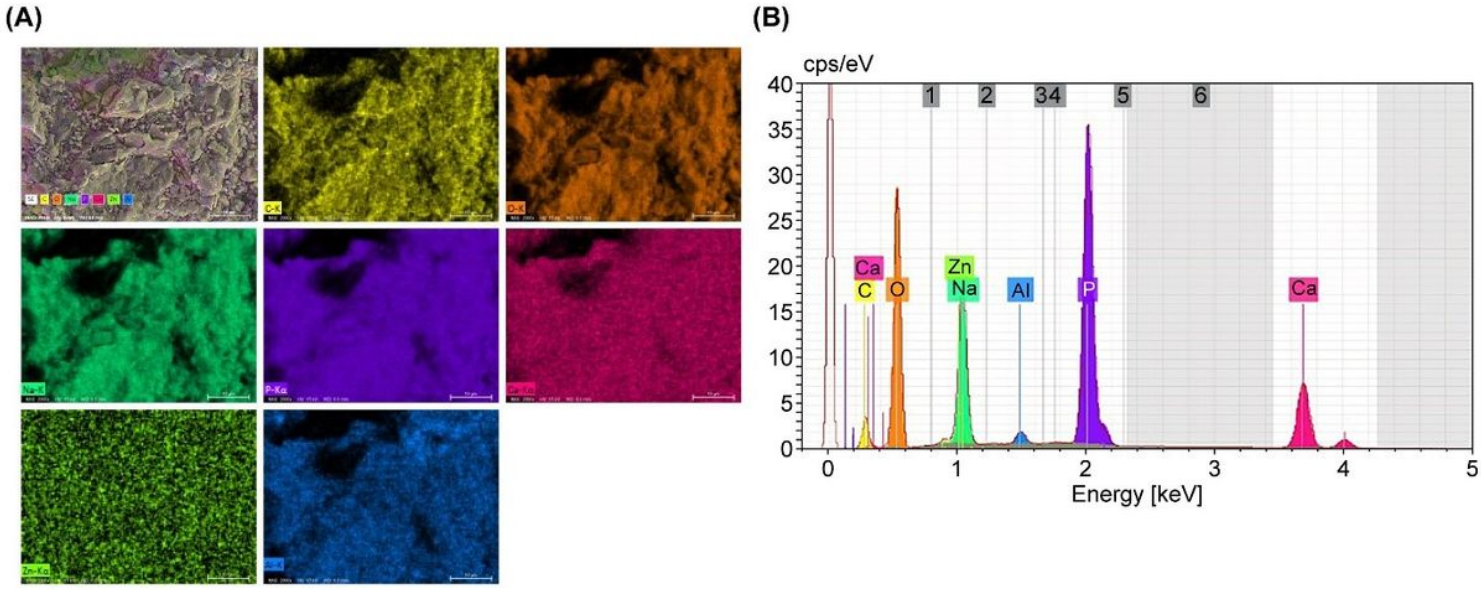

Figure 1

Elemental composition of Zn-PBG powder. (A) Energy-dispersive X-ray microscopy (EDS) mapping images of various elements; (B) EDS profile.

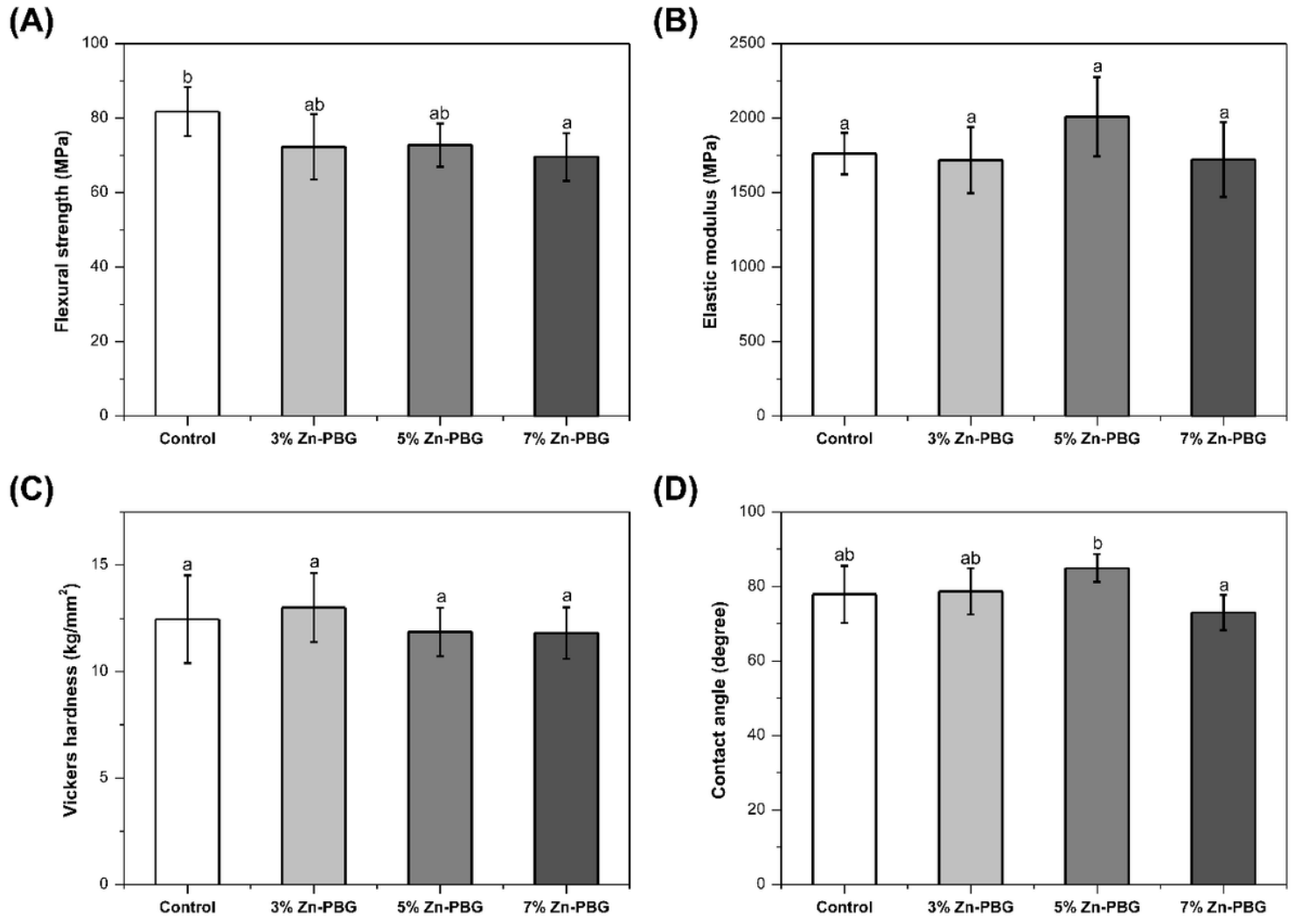

Figure 2

Physical and mechanical properties of different auto-polymerized acrylic resin specimens; each value represents the mean of seven measurements and the error bars show the standard deviation of the mean (mean ± standard deviation; n=7). Different lowercase letters above a bar indicate a significant difference at $p < 0.05$. (A) Flexural strength. (B) Elastic modulus. (B) Microhardness. (D) Contact angle.

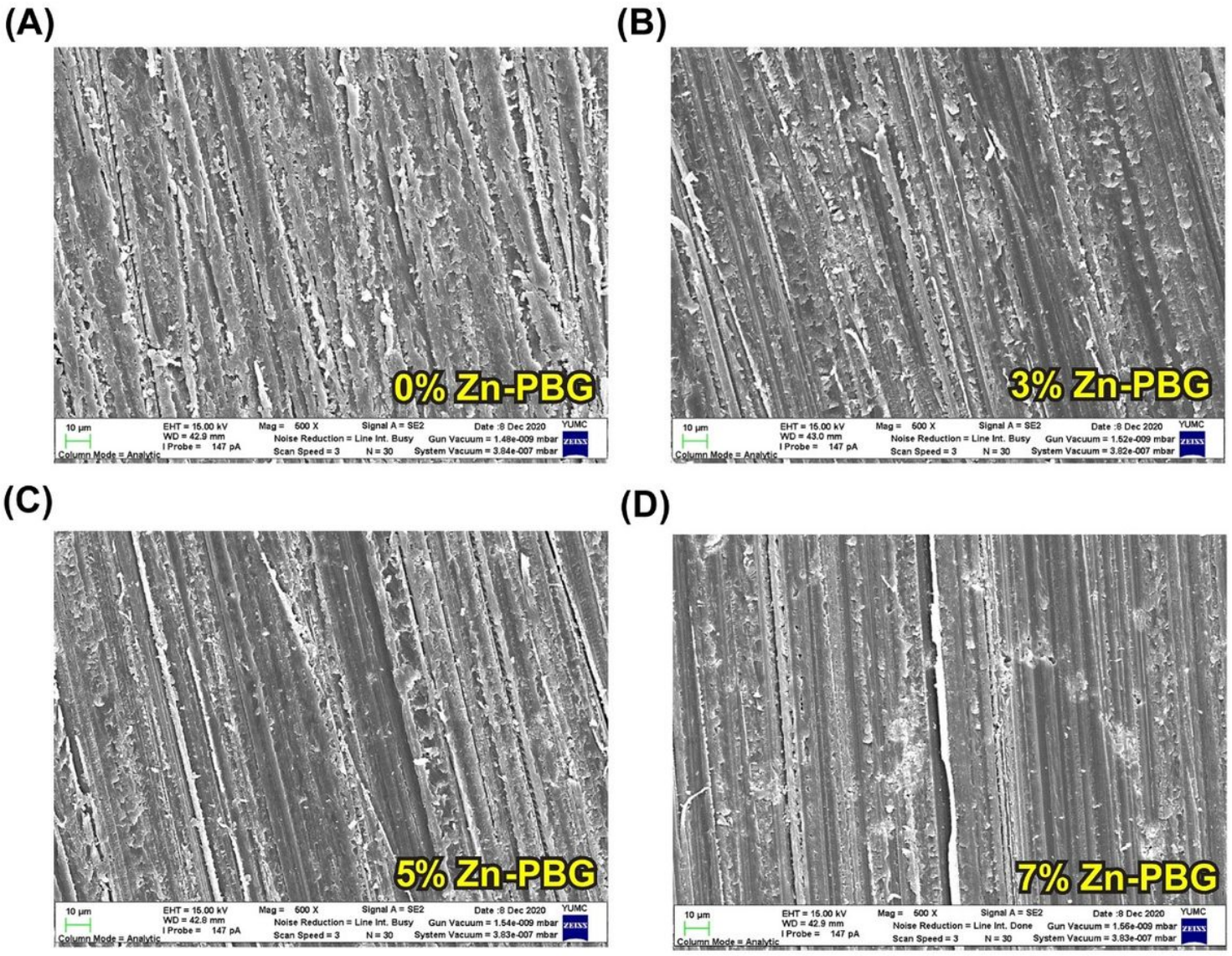

Figure 3

Scanning electron micrographs of surfaces of various auto-polymerized acrylic resin samples used in this study at 500× magnification: (a) control; (B) 3% Zn-PBG; (c) 5% Zn-PBG; (d) 7% Zn-PBG.

Figure 4

Representative scanning electron micrograph**s** of *Candida albicans* on the surfaces of various auto-polymerized acrylic resin samples: (a) control; (b) 3% Zn-PBG; (c) 5% Zn-PBG; (d) 7% Zn-PBG.

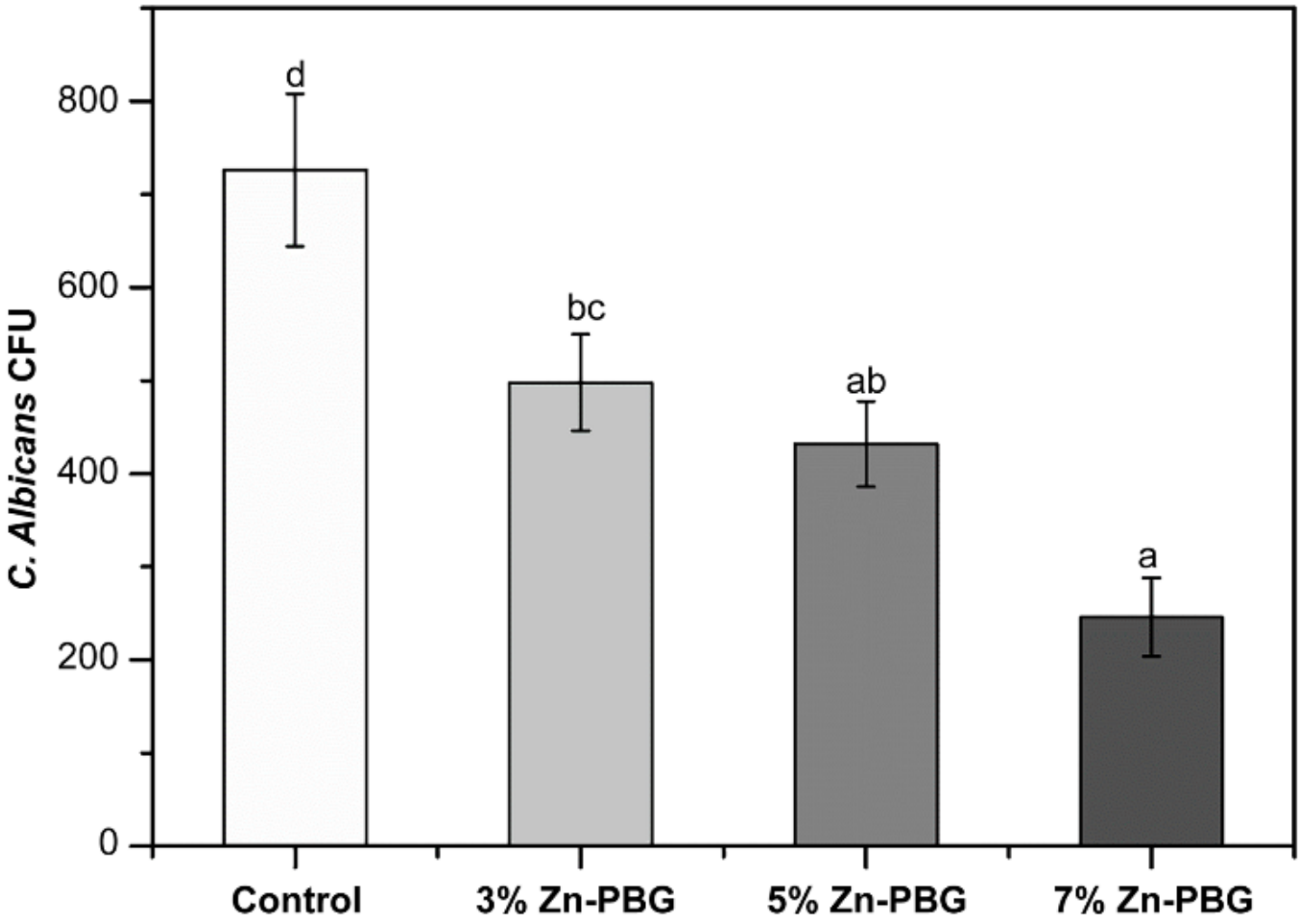

Figure 5

Colony-forming unit counts of fungi on surfaces of various auto-polymerized acrylic resin samples: (a) control; (b) 3% Zn-PBG; (c) 5% Zn-PBG; (d) 7% Zn-PBG. Different lowercase letters above a bar indicate a significant difference at $p < 0.05$.

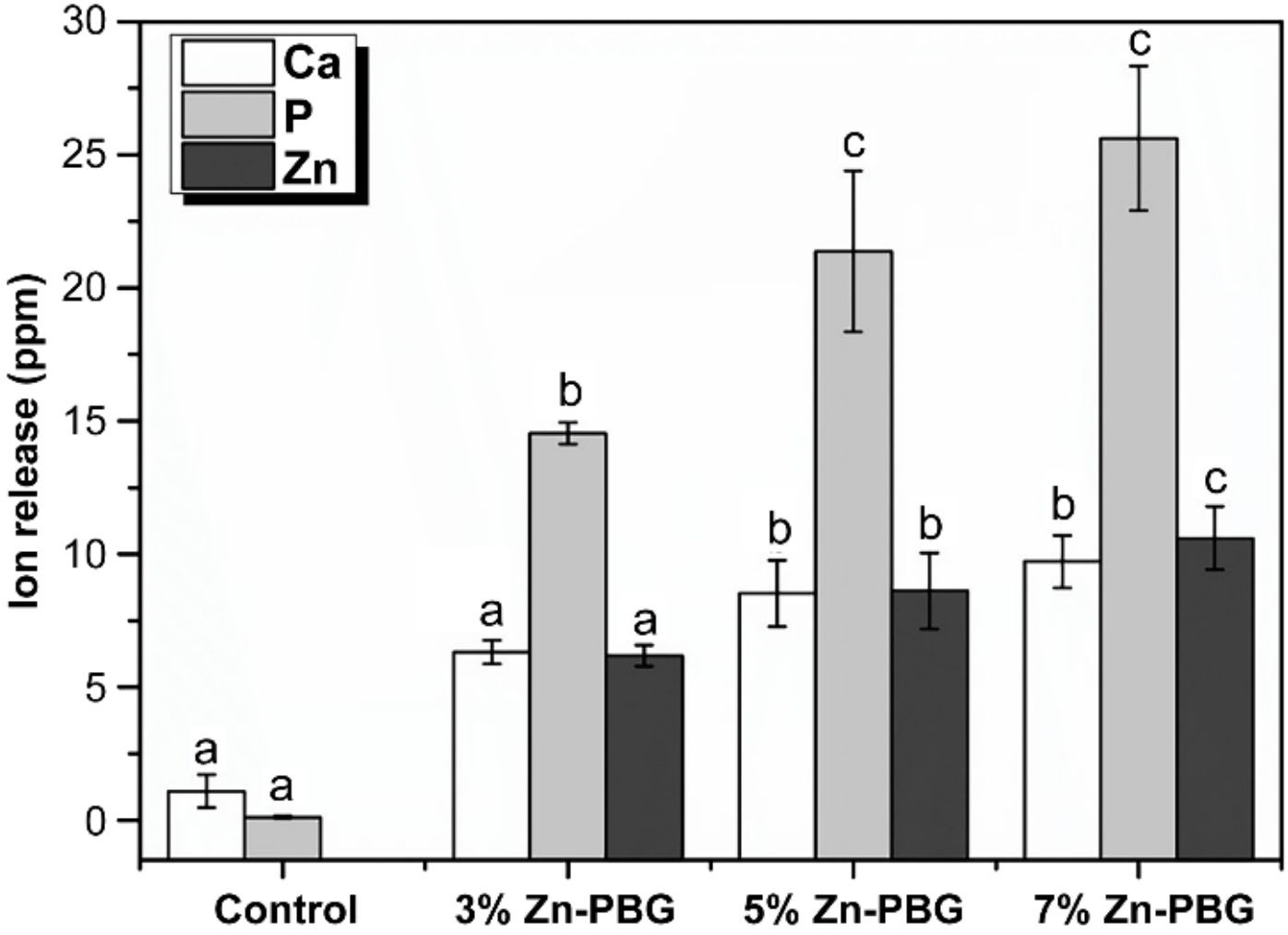

Figure 6

Concentration of ions (Ca, P, and Zn) released from each auto-polymerized acrylic resin sample. The same lowercase letter indicates that there is no statistical difference among the groups with same ion.