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COMPARATIVE EVALUATION OF *CANDIDA ALBICANS* ADHERENCE, FLEXURAL STRENGTH AND WATER SORPTION OF ORTHODONTIC SELF-CURE ACRYLIC RESIN MODIFIED WITH 2-METHACRYLOYLOXYETHYL PHOSPHORYLCHOLINE BY TWO DIFFERENT TECHNIQUES: AN *IN-VITRO STUDY*

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ABSTRACT

Objective: Polymethyl methacrylate (PMMA) is commonly used for removable orthodontic appliances manufacturing. Though, it is liable to fungal and bacterial adhesion. Consequently, this study was conducted to evaluate the antifungal effect, mechanical and physical properties of modified self-curing PMMA resins containing 2-methacryloyloxyethyl phosphorylcholine (MPC).

Materials and Methods. Four groups were evaluated, groups I, II, III were prepared by mixing MPC with the powder at 0 wt% (control), 3 wt%, 4.5 wt%, while group IV was prepared by surface coating method. The surface topography of the resin specimens was investigated using scanning electron microscope (SEM). All specimens were tested for colony forming unit (CFU) count, antibacterial activity, flexural strength, micro hardness and water sorption. The data were statistically analyzed by one-way ANOVA and post-hoc LSD analysis.

Results: All modified groups exhibited high resistance to *candida albicans* adhesion, the highest resistance was recorded for MPC coated specimens with significant difference. No antibacterial property was recorded for all groups. Flexural strength and microhardness of MPC modified specimens showed non-significant change compared to the control group, while there was significant increase of water sorption of MPC coated specimens.

Conclusions: Orthodontic self-cured acrylic resin modified with powder incorporation of 4.5% MPC significantly reduced (CFU) count without any jeopardizing effect on physical and mechanical properties, while surface coating method recorded a significant high water sorption value.

KEYWORDS: *Candida Albicans*, Flexural strength, Water sorption, 2-Methacryloyloxyethyl Phosphorylcholine,

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INTRODUCTION

Removable acrylic orthodontic appliances are designated as retainers following fixed orthodontic treatments to move the teeth and also are used for functional treatments. Their drawback, in spite of their benefits, is the buildup of microbiological plaque on tooth surfaces and regions of acrylic base where retaining components are located.[1] Orthodontic appliances which produce excessive biofilm growth on both tooth surface and retaining spots of acrylic baseplate, making it difficult for patients to practice proper oral hygiene.[2]

The type of resin principally used for fabrication of such orthodontic removable appliances is self-curing acrylic resins, with the composition of PMMA. Satisfactory mechanical properties, biologic compatibility, cost-savings, easy usage, and aesthetic qualities are the main advantages of cold working acrylic resin.[3,4] On the other hand, porosity particularly in thick regions are considered the main drawback of the resin. It is resulted following the reaction of polymerization of polymethyl methacrylate, which relies on the conversion rate of the monomer to the polymer.[5, 6] Because of their lower degree of polymerization, chemically-polymerized acrylic resins exhibit greater porosity compared to heat-polymerized acrylic resins. Presence of porosities produces greater absorption of water, a higher chance of plaque accumulation and bacterial adherence. Microbes including *Candida albicans* and *Streptococcus mutans* can pierce the acrylic baseplate surface by 1 to 2 nm.[7, 8]

Biological properties of PMMA is recommended to be enhanced due to its superior mechanical properties. Modification of the resin matrix by combining nanoparticles is a way that scientists have discovered for improving this material biologically.[9] Orthodontic acrylic baseplate disinfectants and other trials have been made to yield surface modifications that reduced biofilm formation and *Candida albicans* growth. The trials were based on the increase of the hydrophilicity of the surface of the acrylic appliance and thus reducing its energy. All of these factors resulted in poor *Candida albicans* adhesion on the surface. Silicon dioxide, 2-octyl cyanoacrylate, [10] and hydrophilic monomers coated to the surface by graft polymerization created promising results for impeding *Candida albicans* biofilms.[11]

Zwitterionic materials (ZM) contain mutually cationic and anionic groups so maintaining a neutral charge. ZM are considered a class of biologically driven materials that characterized by potent dipole moments and extremely charged groups.[12] One type of zwitterionic materials is 2-methacryloyloxyethyl phosphorylcholine, a methacrylate monomer containing in its lateral chain a polar phospholipid group. It was reported that this material is characterized by high biocompatibility and hydrophilicity, so it has formerly established as an effective protein-repelling and possess anti-adhesion properties.[13]

Previous studies[14, 15]stated that covering the medical devices with a coat of MPC was very beneficial in diminishing adherent bacterial count. As well, there are many applications of the material in various types of dental materials.[14, 15, 16] Most studies investigated the antibacterial and biological effects of modified dental materials with MPC. However, to date limited dental studies assessed the effect of this MPC modification on the mechanical and physical properties of dental materials. So the present study aimed to investigate the method of modification of a self-cured acrylic resin used for orthodontic removable appliances with MPC to produce the highest resistance to C*andida albicans* adhesion without jeopardizing the mechanical or physical properties. The null-hypothesis was that the addition of MPC would not influence the antifungal, antibacterial activity and other properties of orthodontic self-cured PMMA.

MATERIALS AND METHODS

Sample size analysis

Sample size calculated reliant on a previous study[17] as a reference for the Candida albicans adherence test**,** consistent with this study, the minimally conventional sample size was 9 per group, when mean ± standard deviation of in group I was (852 ± 37.5) while the mean ± standard deviation of flexural strength for the control group was (75.4 ± 2.1) MPa [18], the probable mean difference was 3, when the power was 80 % & type I error probability was 0.05. The t test was performed by using P.S. power 3.1.6.

Study design

Our study was in vitro and set experimentally. All steps of our research were approved by the ethical committee of Faculty of Dentistry, Mansoura University with ethical number M0109023 OR. The following groups were prepared: Group I: polymer powder +0% MPC (control), Group II: polymer powder +3% MPC. Group III: polymer powder +4.5% MPC, Group IV: MPC surface coated specimens.

MPC was purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA). Acrostone acrylic resin (Dentsply Sirona, York, PA, USA) was used.

I. Specimens preparations

Modification of the powder: Group II and III were prepared by mixing MPC powder with the polymer powder at mass fractions of 3 and 4.5%.[19] The powder was combined for 10 minutes with a mortar and pestle and measured with a balance (TS4000, Ohaus, Pine Brook, NJ, USA) with a precision of 0.0001 g. Unmodified powder was used for preparation of specimens of both group I and IV.

Preparation of acrylic resin specimens: The powder was mixed with the monomer using the ratio 3:1 by volume until reaching dough stage. Acrylic resin discs were prepared using special molds placed between two metal plates according to each test. The process of polymerization was completed for 10 min following the manufacturing instructions. Specimens were detached from the molds and observed to confirm a smooth flat surface. Specimens containing any defects, voids, or porosity were discarded. After that, the specimens of I, II, III groups were immersed in distilled water at 37 ◦C for 24 h before testing[19], while specimens of group IV were prepared for MPC coating.

Preparation of specimens of group IV: The preparation was done at the Faculty of Science, Department of Chemistry, Zagazig University. Ethanol with 70% concentration was used for the ultrasonic cleaning of the specimens.[14, 20] Cleaning was done for 30 min. The specimens were dried at room temperature then they were inserted in a solution of both ethanol, and 10mg/mL benzophenone for 30 sec. After removal of the specimens, they were dried at room temperature.[14]

Coating of the specimens**:** an aqueous solution of MPC was prepared by liquefying the monomer in pure water at the concentration 0.50 mol/L. Specimens were inserted in the solution of MPC. To complete the photopolymerization process, ultraviolet ray device (White/Ultraviolet Transilluminator TLW-20, UVP, CA, USA) with a wavelength of 365 nm and power of 8 watts was used. Specimens were positioned 20 cm away from the ultraviolet ray source at 70°C for two hours in one step. After achievement of graft polymerization, PMPC-modified acrylic specimens were washed using distilled water to eliminate remaining monomers, and they were dehydrated at room temperature.[14]

II. Scanning Electron Microscopy (SEM)

Surface topography of all groups was investigated by means of Tescan SEM (TESCAN VEGA 3, Czech Republic). Before being analyzed by SEM, specimens were affixed on aluminum stubs and then coated with gold (Au) using Quorum techniques Ltd, sputter coater (Q150t, England).[18]

III. Candida albicans adherence test

The test was accomplished at Department of Microbiology, Faculty of Pharmacy, Zagazig University. Resin disks measuring 2 mm thickness and 6 mm diameter were fabricated. *Candida albicans* was extracted from clinical isolate. Streaking plate method was performed to isolate purified colonies. Specimens were decontaminated by 70% ethanol. Colonies of *C. albicans* were suspended in 5ml saline and the turbidity of suspension was adjusted to 0.5 McFarland. In a new tube, 2ml of Sabouraud dextrose (SD) broth was mixed with specimen that deposited to fill a bottom area of the tube. The mixture was incubated with 100 μL of *candida* suspension for 18hr at 37° c. Specimens were removed and cleaned through phosphate buffered saline (PBS). Each one was put into 1mL of PBS retained in Eppendorf tubes. Vortexed vibration for 2min was performed to isolate attached yeast cells. After immersion in SDA medium, incubation was accomplished at 37 °C for 24 h. The count of attached *C. albicans* to the specimens was calculated, colony forming units (CFU)/mL was used to express the quantity of adherent cells. The contaminated broth was then serially diluted to calculate the number of colonies. Number of colonies/ ml = Number of colonies × dilution factor. [17]

IV. Agar well-diffusion test

Specimens were prepared in a disc shape with a diameter 5 mm and 3 mm thickness. Tested micro-organisms were *Escherichia coli, Candida albicans, and Staphlococcus aureus*, derived clinical isolate, vaccinated by scattering a size of the microbial inoculum (100 µl) over the whole agar surface. A hole with a diameter of 5 mm was punched with a sterile cork borer, resin discs were introduced into the wells, and the agar plates were incubated at 37°C for 48 h. Antibacterial ability of the resin disks were expressed by the diameter of bacterial growth of the inhibition zones which were measured using a scale in millimeters.[21]

V. Flexural Strength

Flexural strength test was achieved following the ISO standard 1567: 1999. Specimens with measurements, 64 mm × 10 mm × 3.3 mm were prepared. Thickness **(d)**, length, and width **(b)** of specimens were measured using a digital caliper. Specimens have been attached to a universal testing machine, (Lloyd Testing Machine, Model 3345; England) a central loading plunger and two supports with a distance 50 mm **(L)**, for three point loading test, at crosshead speed of 5 mm/min. Load **(F)** was directed perpendicular to the middle of each specimen up to fracture expressed in **(N)**. Computer software was used to record the data. $\mathbf{FS = 3FL\backslash 2bd^2}$: the equation that was used for flexural strength **(FS)** estimation in (MPa).[2,18]

VI. Micro hardness test

Using a Teflon mold with a thickness of 2mm and diameter 8mm, disc-shaped specimens were produced. The Digital Panel Vickers Micro-Hardness Tester, (Laizhou Huayin Testing Instrument Co., Ltd. China) was used for assessment of the surface micro hardness. The specimens' surfaces were exposed for 15 seconds to a load of 100g. On every specimen surface, three indentations, with about 0.5 mm distance in between, were made around a circle. The length of their diagonals were recorded through scaled microscope that built in. Micro hardness value estimated following the equation: $\boldsymbol{HV=1.854\ P/d^2}$, **HV** represents the Vickers hardness in Kg/mm^2 and the load is represented by $\boldsymbol{P}$, finally, length of the diagonals symbolized by **d.**[18]

VII. Water Sorption

Specimens with the dimensions, 1 mm thickness and 50 mm diameter, were set using a Teflon mold. Specimens were inserted into dehydrated silica gel

for 1 hour at 37 °C.[22] (Fischer Scientific, Leicester, UK). Weight of each specimen was performed using a precise electronic balance with four numerals. *(Sartorius, Biopharmaceutical and Laboratories, Ger)*. Until the desired weight, also known as the dry weight or the original weight, was reached, weight measurements were repeated. Subsequently, every sample was submerged in deionized water at 37°C ± 1°C within individual containers. Weight changes were used to monitor water sorption and were taken the following week until equilibrium was established (during a period of one week). The weight percent (%) was used to calculate water sorption. To eliminate any visible moisture, samples were taken out of the water, blot dried with filter paper, and then waved in the air for 15 seconds. One minute after the object was removed out of the water, the final weight or weight gained was recorded. The following equation[22] was used for recording water sorption percentage:

$$\text{Water sorption} = \frac{\text{weight gained - original weight}}{\text{original weight}} = 100$$

Statistical Analysis

The minimum, maximum, mean, and standard deviation of each set of data were displayed. with SPSS 16, the statistical analysis was carried out. (Statistical Package for Scientific Studies). Shapiro-Wilk and Kolmogorov-Smirnov tests were used to explore the provided data for normality. The results showed that all of the data were parametric, meaning they were from a normal distribution, and that the significant level (P-value) was insignificant. Relationship between dissimilar groups was done through One Way ANOVA test then Tukey`s Post Hoc test for multiple comparisons was followed

RESULTS

I. SEM

Results of SEM for all groups at 100× magnification are presented in Figure (1). Surfaces of 0, 3, 4.5 wt. % MPC modified specimens exhibited a honeycomb appearance and porous surface. Micrographs showed decreased surface roughness and porosity with increased MPC ratios. Meanwhile, MPC coated specimens demonstrated smoother surface free from porosities.

II. Colony forming unit count and Antibacterial properties

Colonization of C. Albicans was decreased on the modified groups compared with the control one as shown in figure (2). Comparison between all groups regarding candida colonization revealed significant difference between them as $P<0.001$, followed by multiple comparisons which revealed that G1 demonstrated the highest value (659.44 ± 12.51) followed by G2 (120 ± 7.21), then G3 (102.22 ± 5.85), while G4 (54.11 ± 4.08) recorded the lowest significant candida colonization, as presented in table (1) and figure (3). Regarding the antibacterial test, no inhibition zones were recorded for all groups.

III. Flexural Strength and Micro-hardness

Mean and standard deviation recorded values concerning flexural strength (MPa) and Vickers micro hardness number (VHN) (Kg/mm^2)are displayed in Table 1. Graphical representation of the results are shown in Fig. (4) and (5). Statistical analysis revealed no significant differences among all groups, ($P = 0.08$) ns.

IV. Water Sorption

Comparison between all groups regarding water sorption showed significant difference among them as $P<0.001$, followed by multiple comparisons which revealed that group IV (2.54 ± 0.29) demonstrated the highest significant water sorption, while there was insignificant difference between other groups, as displayed in table (1) and figure (6).

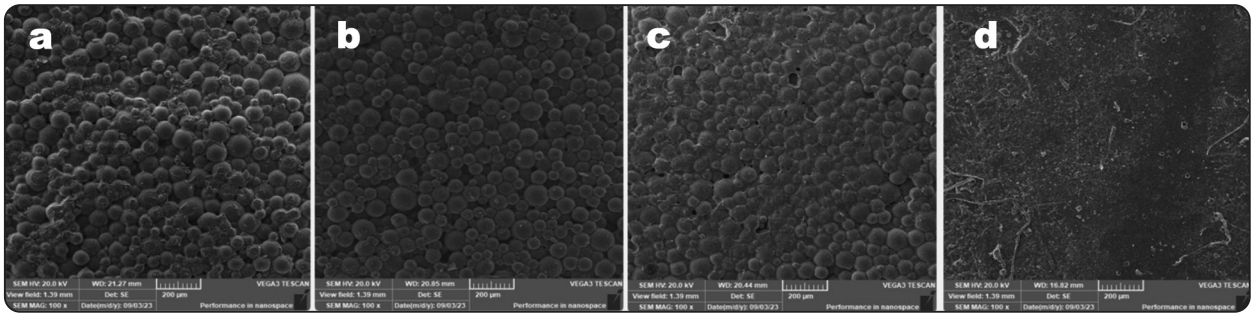

Fig. (1): SE micrograph of acrylic discs: 0% MPC (a), 3% MPC (b), 4.5% MPC (c), and MPC coated discs (d) MPC coated acrylic resin.

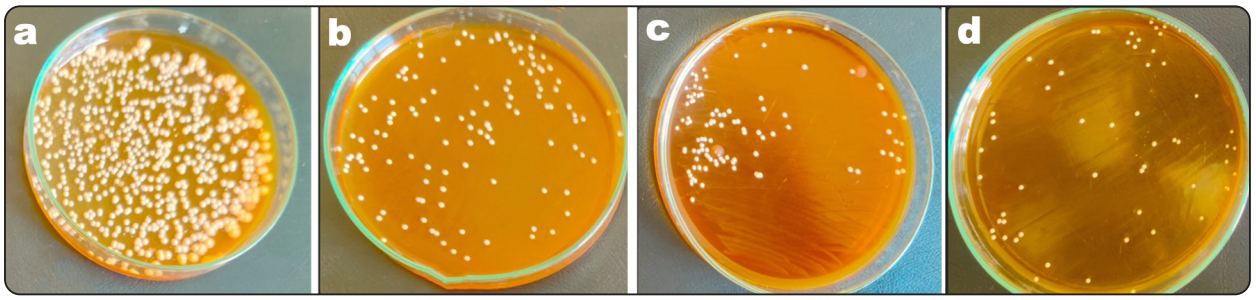

Fig. (2): Colony forming units (CFU)/mL count on SDA medium: a) 0% MPC, b) 3% MPC, c) 4.5% MPC and d) MPC coated discs.

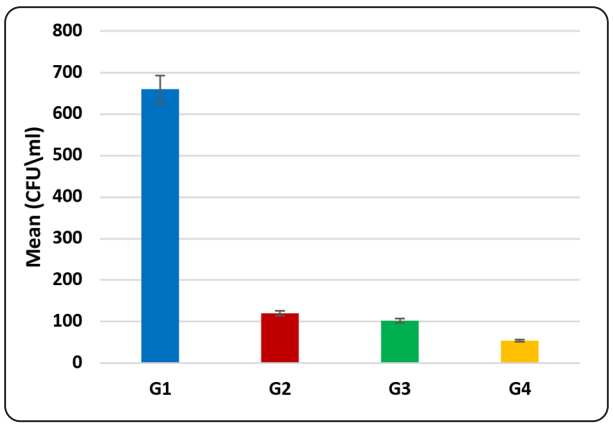

Fig. (3): Bar chart showing candida colonization (CFU) of all groups.

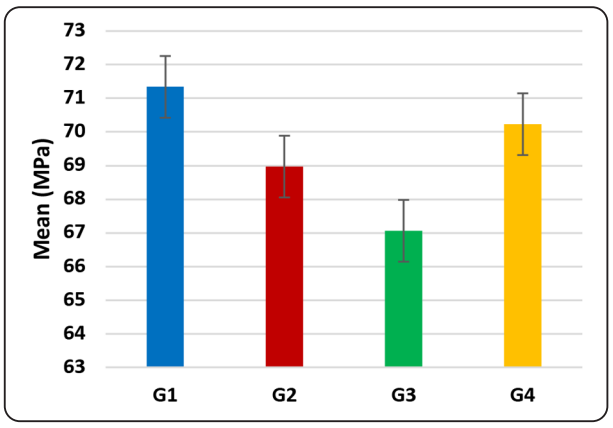

Fig. (4): bar chart showing flexural strength of all groups.

TABLE (1) Mean and standard deviation values of flexural strength, micro-hardness, CFU\mol candida count and water sorption of all groups.

Group	Microhardness (Kg/mm2)	Flexural strength (MPa)	CFU\mol candida	Water sorption %
Gr I	47.67 ± 2.69 [a]	71.34 ±4.02[a]	659.44 ± 12.51[a]	1.21±0.39 [a]
Gr II	49.52 ±3.56 [a]	68.97 ±3.68[a]	120.00 ±7.21[b]	1.42 ±0.67[a]
Gr III	47.73 ± 2.16 [a]	67.06 ±3.07[a]	102.22 ± 5.85[c]	1.31 ±0.33[a]
Gr IV	45.98 ±2.51 [a]	70.23 ±3.25 [a]	54.11 ±4.08[d]	2.54 ±0.29[b]
P value	0.08 ns	0.08 ns	<0.0001*	<0.0001*

significant difference as P<0.05

Means with the same superscript letters were insignificantly different as P>0.05.

Means with different superscript letters were significantly different as P<0.05.

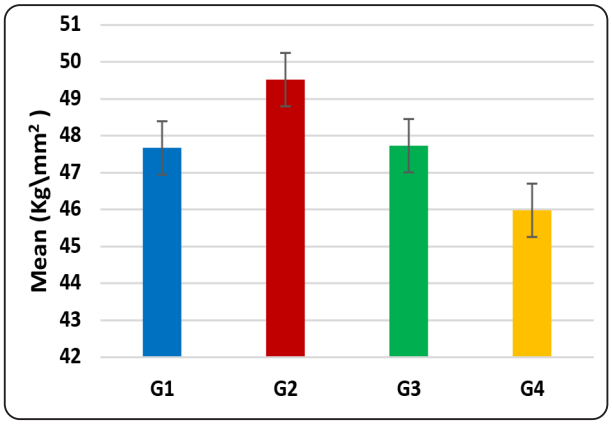

Fig. (5): bar chart showing hardness of all groups.

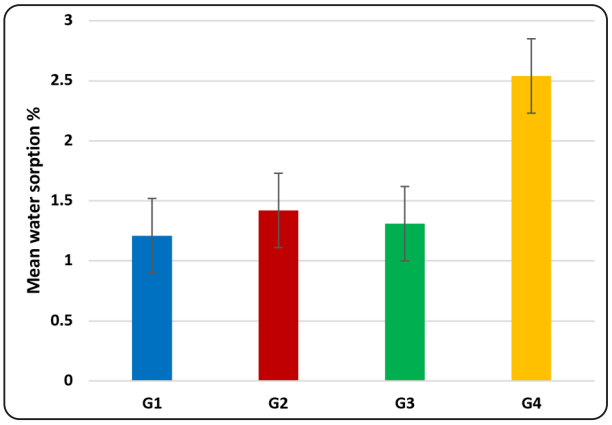

Fig. (6): bar chart showing water sorption percentage of all groups.

DISCUSSION

The diagnosis and treatment of maxillofacial anomalies through orthodontics are crucial in determining the craniofacial area's morphology, function, and appearance in both children and adults. When treating dental and skeletal issues in children, removable orthodontic appliances are frequently utilized. Both the patient and the dentist may easily place and remove these appliances, which are also manufactured in a laboratory using dental casts. The majority of the day and night must be spent with removable appliances in the mouth. As the appliances taken out for eating and cleaning, an environment for the growth of different oral bacteria was formed.[23] These microbes may be responsible for the malodor that emanates from orthodontic base plates, which has a detrimental effect on patient participation. Chronic atrophic (erythematous) candidiasis, is defined by mucosal edema and limited persistent erythema that contact the appliance fitting surface.[24] Its primary cause is the potential for candida to overgrow in the area where natural salivary flow is restricted, which is between the palate and the appliance surface.[25]

Many techniques were used to modify acrylic resin with MPC polymer. Previous research[19] added MPC monomer in various ratios to polymer powder while other performed MPC surface modification.[14,26] Among the often employed techniques are graft polymerization, plasma treatment, and substrate surface coating.[27] Because of the chemical interactions that bond both polymer and denture base resin, constant modified surfaces by MPC was yielded as a result of the graft polymerization reaction.[14] Our study modified cold cure acrylic resin by MPC monomer using two method, surface coating and powder modification with different ratios. The selected modified ratios were 3% and 4.5% based on the most common concentrations considered in the earlier studies.[13,14,19] It was established that ratio below 3 wt.% MPC could cause insignificant resistance to fungal adhesion while ratio above 4.5 % MPC cause detrimental effect on the properties of the resin. MPC concentration of 0.50 mol/L was prepared for specimens coating. A previous study[17] stated that this concentration was enough for PMMA surface modification to resist *Candida albicans* adhesion.

Scanning analysis of the unmodified specimens exhibited a honeycomb form containing an irregular and porous construction. Specimens of modified powder exhibited the same structure but with decreased porosity. The finding is in agreement with a previous study[18]. The explanation is due to agglomerations of pre-polymerized PMMA globules

enclosed by in-situ PMMA that was created by the polymerization reaction of methyl methacrylate monomer liquid. The scanning micrograph of MPC coated samples presented a homogenous non-porous structure. The result is attributed to the chemical bond that formed between MPC and the resin yielding smooth non porous coat on the surface.

The present study examined the ability of MPC to combat *Candida albicans* adhesion through colonies forming units (CFU) counting on plates. This method was chosen due to their advantages as any number of bacteria can be count either too many or too few concentrations using dilutions. Additionally, viable bacteria are counted only discounting any debris and dead bacteria. The results of this study showed significant reduction of *C. albicans* adhesion following increased concentrations of MPC for both modified methods. The result is in agreement with previous studies [14, 19] which stated that 4.5 % MPC exhibited low *Candida* adhesion compared to 3% MPC modified powder. The result is explained by the fact that *Candida albicans* adhesion increased with hydrophobic surface. MPC modified groups accepted high rise in the hydrophilicity based on the structure of MPC containing a phospholipid polar group. The structure of phospholipid molecules including two parts, one that attracted to water, hydrophilic head and other part that resisted by water, hydrophobic tail.[28] The phosphorylcholine group of the MPC polymer would be exposed to a significant amount of free water due to its unique structure. The hydrated MPC would not have any bound water, which would encourage protein adsorption while a high level of free water would draw away proteins.[29]

Surface coated specimens recorded lower *Candida* count than powder modified ones. According to a prior studies [17,30] demonstrated MPC surface coated on acrylic and polyamide resin, found that method of surface coating recorded highly significant decrease in *Candida* count compared to the unmodified one. This new technique produced variations of efficient grafted particles by fixing several monomers to hard surfaces. This method's idea is based on establishing a system of redox surface at the interface between both the solution and particles. Consequently, a great number of free radicals are generated on surface of solid particles, and the monomer readily begins to polymerize on the particles, resulting in better strength and well-organized function.[31]

Regarding the antibacterial test considering the bactericidal effect of the material, none of the specimens either control or modified ones, resulted in the formation of a zone of inhibition. The finding was not in accordance with the reduced CFU counts on modified resins. The result was in agreement with those of previous study[29] demonstrating that the antibacterial effect of MPC resulted from prevention of bacterial or fungal attachment via protein-repellent properties. This property of MPC resulted from its hydrophilic effect as it has no bactericidal ability.

Flexural strength of any material reproduces its probability to struggle sudden failure when subjecting to a flexural load. The clinical success of the acrylic restorations is achieved by its high flexural strength, assumed the information that the alveolar resorption occurs in a steady, uneven manner yielding uneven maintenance of tissue-borne prostheses.[32] Important factors that have significant influence on the flexural strength are high water sorption and solubility of acrylic resins.[33] There was non-significant reduction of flexural strength for MPC modified resin compared to the control one. The obtained values surpass the lowest requisites of flexural strength that is not fewer than 50 MPa.[34] The results are coincide with a previous study[35] which explained the finding by the fact that MPC polymer was consistently blended within the polymer matrix. This ideal combination produced

interactions either chemically or physically between MPC polymer and PMMA.

Surface hardness of removable orthodontic appliance is a primary feature to combat its scratching on clinical service and consequently food, bacterial and fungal accumulation. The bond of these microbes to the hard surface is avoided with consequent candidiasis and inflammation.[36] In this study, no significant differences were recorded regarding the micro hardness values. The results may be attributed to the low concentration of the homogenously blended MPC particles within the polymer matrix so no negative effect was recorded for MPC modifications.

Polar carbonyl groups were included in the composition of acrylic resin that consequently draw water molecules that enter among the polymer intermolecular gaps splitting them a little and progressively intrude into deep parts of the resin.[37] This composition of acrylic resin material permits specific degree of water sorption. Water molecules seep in and function as a plasticizer, influencing the base's longevity and dimensional stability.

The ADA specifies a maximum water sorption of 0.8 mg/cm^2, but the Institute of Standards and Industrial Research of Iran and ISO list a maximum value of 32 μg/mm^3.[38] The percentage of water sorption was used to assess the water sorption in our study. The results revealed no significant differences of water sorption percentage between the control, 3% MPC, and 4.5% MPC specimens. While MPC coated specimens recorded significant increase of water sorption. The results is in agree with a previous study stated that high concentrations of MPC are not ideal in terms of maintaining water sorption value of calcium silicate-based cements.[29] The fact that 2-methacryloyloxyethyl phosphorylcholine (MPC) is a hydrophilic methacrylate with a phospholipid polar group helps to explain this outcome.[17, 19] MPC surface coated produces more hydrophilicity than MPC incorporated in between the particles of the polymer. Consequently, water freely adsorbs to the surface which is hydrophilic by hydrogen bonds formation with the substrate. Furthermore, the mechanism of water uptake by methacrylate-based resins is enhanced by hydrogen bonding with pre-adsorbed water molecules. The polarity of these resins defined by their Hoy's solubility values for polar forces is positively connected with their capacity to absorb water.[39, 40]

CONCLUSIONS

1. Both modification methods of orthodontic acrylic resin with MPC recorded an inhibiting effect on *Candida albicans* adhesion, without any significant effect on mechanical properties.
2. Modified MPC powder has no effect on the water sorption, while MPC coated specimens exhibited high water sorption compared to other groups.
3. MPC has no bactericidal effect as no zones of inhibition of bacterial growth were recorded for any group.
4. Modified orthodontic resin with 4.5 % MPC is very promising formulation that exhibited high resistance to *Candida albicans* adhesion with an acceptable mechanical and physical properties of the resin.

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