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DEVELOPMENT OF A NOVEL REMOVABLE ACRYLIC ORTHODONTIC APPLIANCE MATERIAL WITH PROTEIN - REPELLENT AND ANTIFUNGAL PROPERTIES

Marwa Sameh Shamaa*, Sayed Mohamed Ghorab** *and* Nehal Fouad Albelasy***

ABSTRACT

Objective: To develop an innovative self-cure orthodontic acrylic resin containing 2-methacryloyloxyethyl phosphorylcholine (MPC) and explore the impact on protein-repellent capacity, antifungal activity, surface roughness and flexural strength.

Materials and Methods: MPC was added to polymethyl Methacrylate PMMA resin in three different concentrations forming four groups (0 [control], 1.5, 3, and 4.5%). The protein adsorption was assessed utilizing a micro bicinchoninic acid method. Candida albicans biofilm activity was estimated via colony forming unit counts. Surface roughness was evaluated utilizing a Mitutoyo surface roughness tester. Flexural strength was tested in three-point flexure utilizing a Universal Testing Machine. Data were statistically analyzed using ANOVA and Tukey HSD tests ($\alpha = 0.05$).

Results: Incorporating MPC into the self-cure orthodontic acrylic resin significantly reduced both protein adsorption and C. albicans CFU compared to control group ($p < 0.001$). Adding 4.5 wt% MPC to the self-cure orthodontic PMMA resin raised the roughness values significantly ($p = 0.012$), while adding 1.5% and 3 % MPC resulted in no difference in roughness values to that of the control group ($p > 0.05$). The incorporation of 3 wt% MPC into PMMA resin significantly increased the flexural strength ($P < 0.05$). However, PMMA resin incorporating 4.5 wt% MPC revealed significant reduction in flexural strength compared with the control group ($p = 0.009$).

Conclusion: A novel removable acrylic orthodontic appliance material incorporating 3 wt% MPC could achieve a promising protein repellent and antifungal activity without adversely affecting the surface roughness and flexural strength of PMMA resins.

KEYWORDS: protein repellent; Candida albicans; flexural strength.

* Associate Professor, Orthodontic Department, Faculty of Dentistry, Mansoura University
** Associate Professor, Dental Biomaterials, Faculty of Dentistry, Mansoura University
*** Lecturer in Orthodontic Department, Faculty of Dentistry, Mansoura University

INTRODUCTION

Nowadays there is increased demand for orthodontic treatment not only for correction of malocclusion but also to enhance general oral health and facial esthetic.[1] Orthodontic appliances can be removable or fixed or semi-fixed. Removable orthodontic appliances are widely utilized for simple tooth movement, as a mean of interceptive treatment and for retention after orthodontic mechanotherapy. Inspite of their benefits, usually aggregation of plaque occurs on the dental surfaces, their retentive components and the acrylic base plate. [2]

Self-curing acrylic resins, primarily consisted of polymethyl Methacrylate (PMMA), have been extensively utilized in the fabrication of orthodontic removable appliances, because of their favorable characteristics like biocompatibility, convenient mechanical properties and desirable esthetics. [3]

Researches have revealed that self-polymerizing acrylics exhibit higher porosity than heat-polymerizing ones due to their lower degree of polymerization. Thus, self-curing acrylic resins are more likely to absorb water than other resins and are more prone to microbial adherence and plaque aggregation resulting in high incidence of caries and periodontal diseases. [4, 5]

It has been observed that nightly wear of the removable orthodontic appliances can contribute to high prevalence of infection with oral candidiasis. There are several factors supporting Candida colonization and multiplication underneath acrylic resin surface. These include the diminished salivary flow and subsequently lower pH values, rough surface texture of acrylic, along with compromised oral hygiene.[6] These appliances also provide a hydrophobic surface that C. albicans attach to and cover a large surface of the oral mucosa for a substantial amount of time every day for a reasonably prolonged duration and so provide a better atmosphere for the growth and multiplication of C. albicans.[7]

Different treatment approaches have been suggested for treatment of C. albicans 'infections related to acrylic removable orthodontic appliances. Some of these strategies involve utilizating antifungals like miconazole and nystatin, and disinfectants like hydrogen peroxide and chlorhexidine gluconate.[8] Other approaches have been introduced including surface modulation of the acrylic resin to decrease the candidal adherence and biofilm development. They enhance the acrylic resin's surface hydrophilicity and decrease its surface energy, which lead to decreased candidal adherence and accumulation on the appliance surface. Examples of these substances are silane-silicon dioxide (SiO_2) and 2-octyl cyanoacrylate, which showed convenient results in preventing candida biofilm formation. [9]

Protein adsorption is a primary necessary stage in microbial engagement to a surface, which prepares anchor sites for fungi attachment to the substance surface.[10] Thus, it would be recommendable to form a noval poly methyl Methacrylate resin which could repel proteins and decrease the microbial adherence. 2-methacryloyloxyethyl phosphorylcholine (MPC) is a methacrylate possessing a phospholipid polar group in its side chain. It has exhibited efficient protein repellent and antiadherence charateristics owing to its hydrophilicity and biocompatibility. [11] Formely, MPC was added to dental adhesives and it showed a significant decrease in protein repellence and microbial colonization without affecting their mechanical features. [12]

Till now, there is no data concerning the incorporation of MPC into self-cure orthodontic acrylic resin material and the propable advantages of its utilization in preventing oral candidiasis. Therefore, the objective of the present work was to explore the influence of adding several concentrations of MPC into self-cure orthodontic acrylic resin on protein adsorption, candida albicans biofilm adherence, surface roughness and flexural strength. It was hypothesized that incorporation of MPC into PMMA resin would: (1) cause less protein adsorption than the control group without MPC; (2) decrease the colony-forming unit counts in comparison with the control group; (3) not compromise the surface roughness; and (4) not jeopaeadize the flexural strength compared to the control without MPC.

MATERIALS AND METHODS

Materials

An auto-polymerizing orthodontic acrylic resin (Orthocryl, Dentaurum GmbH& Co., Ispringen, Germany) was used in this study as the carrier for the protein-repellent monomer, 2methacryloyloxyethyl phosphorylcholine (MPC). The compositions, batch numbers and manufacturer of these materials are shown in Table 1.

TABLE (1) Materials utilized in the studyv

Material	Composition	Batch no	Manufacturer
Orthocryl (Self-polymerizing orthodontic acrylic)	**Powder:** Polymethyl methacrylate **Liquid:** Methyl methacrylate, ethylene glycol dimethacrylate, N,N dihydroxyethyleneP-toluidine	417920 A	Dentaurum GmbH & Co., Isprengen, Germany.
MPC (Protein repellent material)	2-Methacryloyloxyethyl phosphorylcholine	MKCG3377	Sigma- Aldrich, Chemie GmbH, Steinheim, Germany

Methods

Preparation of MPC containing acrylic resin material

A digital electric balance (HR-202, A&D Instruments, India) with four digits accuracy was utilized for weighting the 2-Methacryloyloxyethyl phosphorylcholine powder to be added in three different ratios (1.5, 3 and 4.5 wt%) to acrylic resin and mixed separately to form four experimental groups; Group 1: control (0 wt% MPC), Group 2: 1.5 wt% MPC, Group 3: 3 wt% MPC and Group 4: 4.5 wt% MPC. The mixture was blended with electric mixer at a rotating speed of 400 rpm at room temperature for 30 min to achieve an equal and homogenneous distribution of MPC powder.

Specimens' preparation

A total of one hundred and sixty specimens were prepared in this study. Sample size was calculated using G power program version 3.1.9.4 based on effect size of 1.40, level of alpha error of 5% and study power of 85% retrieved from a previous research (Bajunaid et al., 2021). [8] A minimal sample size required for the study was calculated to be 10 samples for each group. The research protocol was approved by the Ethical Committee, Faculty of Dentistry, Mansoura University (No. A0103024OR). For protein adsorption, C. albicans biofilm and surface roughness testing: one hundred and twenty disc-shaped samples (ten samples per each group) were prepared utilizing stainless steel molds (8 mm in diameter and 0.5 mm in thickness). For flexural strength testing, forty rectangular shaped samples (ten samples per each group) were prepared utilizing stainless steel molds (2 × 2 × 25 mm).

Using these molds, spaces with the same dimensions were created in condensation silicone rubber base material. The monomer polymer ratio (2.5/1 g.ml-1) was mixed, loaded in mold spaces, and compressed utilizing glass slabs by hand pressure to get rid of excess material and air bubbles in accordance with the manufacturer's instructions.

A plastic sheet was utilized to separate the glass slabs from the filled molds. Following polymerization for five min, the samples were removed from mold spaces, smoothed and polished using 800-grit SiC papers (Struers Ballerup, Denmark) (Figure 1). The dimensions of each sample were assessed utilizing a digital caliper (Hogetex, Germany) with 0.01 mm precision.

Samples were kept in distilled water at 37 degree Celsius for twenty four hours before testing.[13]

Measurement of protein adsorption

The amount of protein adsorbed on the samples was estimated utilizing the micro bicinchoninic acid (BCA) method. Each disk was submerged in phosphate buffered saline (PBS) for two hours before immersion in 4.5 gram per litre bovine serum albumin (Sigma-Aldrich, ChemieGmbH, Steinheim, Germany) suspensions at 37 degree Celsius for two hours. The disks were then washed for five minutes with fresh PBS by blending at a speed of 300 rpm after submersion in sodium dodecyl sulfate at 1 wt% in PBS. To separate the bovine serum albumin adsorbed on the sample, they were sonicated at room temperature for twenty minutes. A protein assessment kit (micro bicinchoninic acid protein assay kit, Pittsburgh, PA, USA) (Figure 2) was utilized to estimate the bovine serum albumin value in the sodium dodecyl sulfate solution. Calculation of the quantity of adsorped protein was done according to the protein concentration.[14]

C. albicans biofilm adhesion

Colony Forming Unit (CFU) counts test was used to measure C. albicans biofilm adhesion on acrylized disk-shaped specimens. It was conducted at the Medical Microbiology and Immunology Department, Faculty of Medicine, Mansoura University.

A dental plaque microcosm biofilm model utilizing human saliva was utilized to test the protein-repellent resins. Saliva is perfect for growing microcosm biofilms in vitro, due to its capability of preserving the heterogenous features of the plaque in vivo.[15] Collection of saliva was done from 10 healthy donors with sound dentition with no history of taking antibiotics in the preceding three months. The donors received instructions not

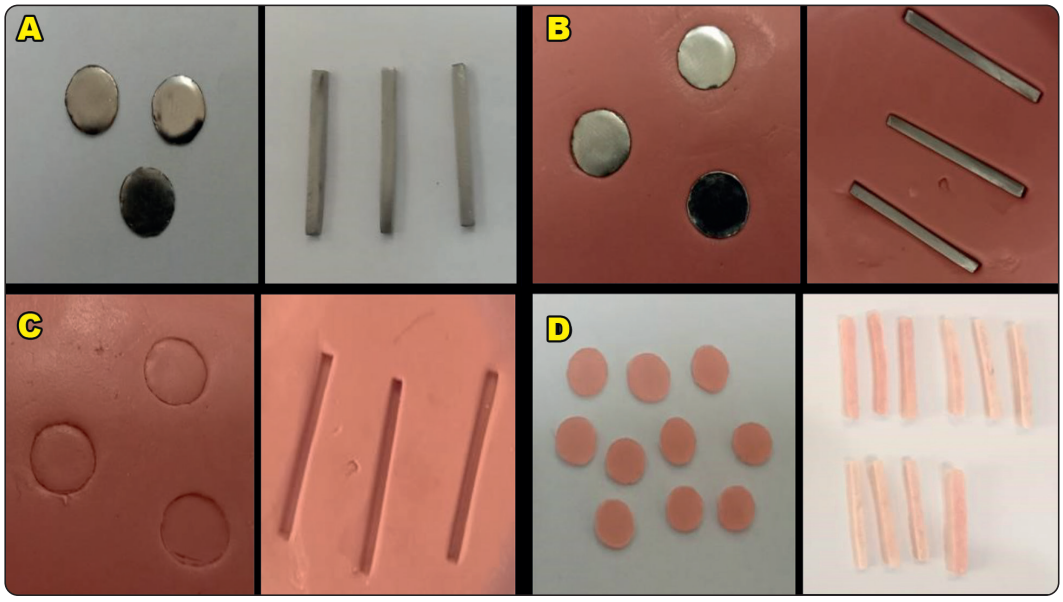

Fig. (1) Steps of preparation of the acrylic sample (A) Stainless steel molds with various dimensions for each test (B) Using stainless steel mold in rubber base to make space with similar dimensions, (C) Space for self- cure acrylic insertion, (D) Acrylic specimens of different dimensions utilized in this study.

Fig. (2) Fisher Scientific micro (BCA) protein assay kit used in this study.

to make brushing for twenty four hours and not to eat or drink for two hours before salivary collection. Stimulated saliva was collected during parafilm chewing and was retained in ice. A similar volume of saliva from every participant was combined to form the salivary sample. Each disc was sterilized with ethylene oxide (STERRAD® 100S System, Soma Tech Intl, Espanol). The saliva was diluted with glycerol to reach a conc of 70% saliva, and preserved at –80° C for next use.

The saliva-glycerol stock was inserted, with 1:50 dilution, into the McBain growth medium as inoculum. Then, 1.5 mL of inoculum was inserted into every well of 24-well plates comprising a resin disc and incubation was done all-night at 37 degrees Celesius in 5% carbon dioxide incubator (Heraeus B 5060 EK-CO2, Analytical Instruments LLC, USA). Afterwards, the discs were transmitted to noval 24-well plates full of fresh media and incubated. After sixteen hours, the discs were transmitted to noval 24-well plates with fresh medium and incubated for one day. This overall 48 hours of incubation was appropriate to develop plaque microcosm biofilms. [16]

Colony Forming Unit (CFU) counts

Two-day biofilms were added to tubes containing 2ml cysteine peptone water. Harvesting of biofilms was done by sonication (3510RMTH, Branson, Danbury, CT) for 5 minutes, then vortexing (Vortex Mixer VM-300, Gemmy Industrial Corp., Taipei, Taiwan) at 2400 rpm for 30 seconds. Sabouraud Dextrose Agar (SDA) is a perfect media mainly utilized for identification of fungi, yeasts, and filamentous bacteria. The pH of the media is about 5.0. This acidic pH prohibits the bacterial growth but allows yeast and most filamentous fungi growth. Sabouraud Dextrose Agar was utilized to assess candida albicans. The solutions were sequently diluted, added to agar plates for incubation at 37 degree Celsius in 5% carbon dioxide for 1 day (Figure 3). Counting of the growing colonies was done and utilized with dilution factor to estimate the total colony forming unit counts on every disc. [17]

$$\text{CFU/ml} = \frac{(\text{no. of colonies} \times \text{dilution factor})}{\text{volume of culture plate}}$$

Measurement of surface roughness

A profilometer (SURFTEST SJ-201, Mitutoyo Corp., Kawasaki, Japan) was utilized for estimation of the surface roughness.

Five readings were registered for every sample and the mean roughness value (Ra) of the sample was estimated. The cut-off length was 0.8 mm, at 0.5 mm/s scanning speed. The resolution of the registered data was 0.01 μm. [18]

Flexural strength testing

Assessment of the breaking strength of the samples was done utilizing A computer-controlled Universal Testing Machine (Model 3345, Instron Corp, England) with a crosshead speed of 1mm/min and three-point flexure with a span of 22 mm (Figure 4). Assessment of the flexural strength (S) was done as: $S = 3P_{max}L/(2bh^2)$, where P_{max} is the maximum load on the load–displacement curve, L is the flexure span, b is the sample width, and h is the sample thickness. [19]

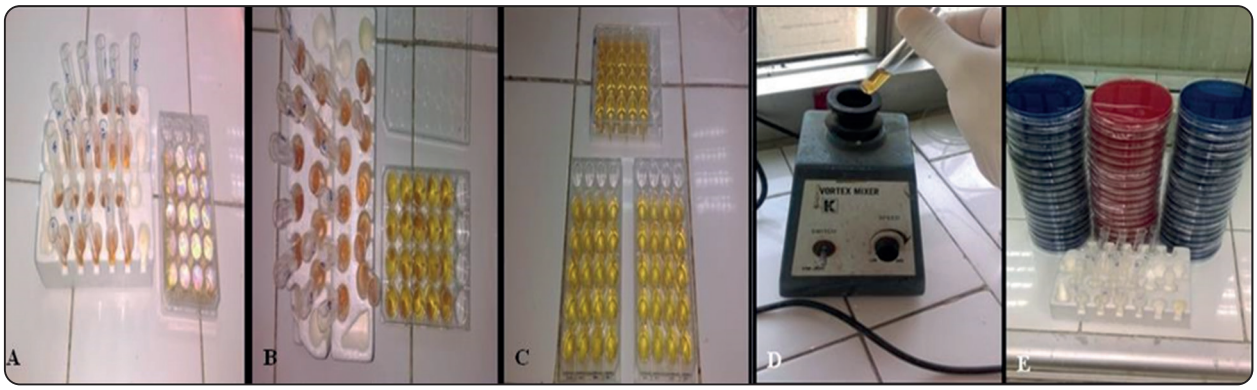

Fig. (3) Steps for dental plaque microcosm biofilm testing. (A) Resins discs in 1.5 mL of inoculum were incubated at 37 degree Celsius in 5% CO2 overnight, (B) Transferring resin discs in fresh medium for 16 hours incubation at 37 degree Celsius in 5% CO2, (C) Repeating former step for 24 hours incubation at 37 degree Celsius in 5% CO2, (D) Transferring discs into tubes with two mL CPW, and vortexing at 2400 rpm for thirty seconds utilizing a vortex mixer, (E) The solutions were diluted, added to Sabouraud Dextrose Agar plates for incubation at 37 degree Celsius in 5% CO2 for 24hours.

Fig. (4) Specimen mounted on Universal Testing Machine for three-point flexure test.

Statistical analysis

Data were tabulated, and analyzed utilizing SPSS program (SPSS v25.0; IBM Corp.). Test of normality was carried out utilizing Shapiro Wilk test and homogeneity of variances using Levene's test. The data were normally distributed and presented as mean ± standard deviation (SD) for descriptive statistics. One Way ANOVA was utilized to estimate the effect of different MPC wt% concentrations in every group accompanied by Tukey's multiple comparisons if significant differences were determined. P was significant at 5%.

RESULTS

Protein adsorption

A comparison of protein adsorption (ug/cm^2) among different groups is presented in Table 2 and Figure 5. There was a significant difference in protein adsorption among the experimental groups ($P < 0.001$) detected by one-way ANOVA test. Group 1 recorded the highest mean protein adsorption value (9.52 ± 0.509 μg/cm^2), while group 4 recorded the lowest value (2.23 ± 0.018 μg/cm^2). Tukey HSD statistical test showed a significant reduction in all groups compared to control group (group 1), and in group 3 and group 4 compared to group 2 and in group 4 compared to group 3.

C. albicans biofilm

A comparison of C. albicans biofilm (CFU/Disk) among different groups is presented in Table 2 and Figure 6. There was a significant difference in protein adsorption among the experimental groups ($P < 0.001$) identified by one-way ANOVA test. Comparing the mean C. albicans biofilm of the tested groups; group 1 exhibited the highest value (74635.3 ± 2155.5 CFU/Disk), while group 4 showed the lowest value (5530.0 ± 245.1 CFU/Disk). Tukey HSD statistical test displayed a significant reduction in all groups compared to control group (group 1),

and in group 3 and group 4 compared to group 2 and in group 4 compared to group 3.

Surface roughness

A comparison of surface roughness (μm) among different groups is showed in Table 2 and Figure 7. One-way ANOVA test showed that there was a significant difference in surface roughness among the experimental groups (P = 0.002). Group 4 recorded the highest mean surface roughness value (2.36 ± 0.273 μm), while the lowest value was for group 1 (1.58 ± 0.474 μm). Tukey HSD statistical test showed a significant elevation in group 4 compared to both control group and group 2. Otherwise, no other significance could be detected.

Flexural strength

A comparison of flexural strength (MPa) for all groups is displayed in Table 2 and Figure 8. There was a significant difference in flexural strength among the experimental groups (P < 0.001) detected by One-way ANOVA test. Comparing the mean flexural strength of the tested groups; group 3 showed the highest value (46.75 ± 15.34 MPa), while group 4 exhibited the lowest value (16.15±2.75 MPa). Tukey HSD statistical test revealed a significant reduction in group 4 compared to other groups. On contrary, there was a significant elevation in group 3 compared to both group 1 and group 4. Otherwise, no other significance could be detected.

TABLE (2) Comparison of Protein adsorption (μg/cm2), C.Albicans biofilm (CFU/Disk), Surface roughness (μm) and Flexural strength (MPa) among studied groups.

	Group 1 (Control) (0.0 wt% MPC)	Group 2 (1.5 wt% MPC)	Group 3 (3.0 wt% MPC)	Group 4 (4.5 wt% MPC)	One-Way ANOVA (P-value)
	X ± SD	X ± SD	X ± SD	X ± SD	
Protein adsorptin (μg/cm²)	$9.52^{d} \pm 0.509$	$7.67^{c} \pm 0.467$	$3.81^{b} \pm 0.049$	$2.23^{a} \pm 0.018$	<0.001
C.Albicas biofilm (CFU/Disk)	$74635.3^{d} \pm 2155.5$	$58686.6^{c} \pm 4799.5$	$17940.6^{b} \pm 2173.1$	$5530.0^{a} \pm 245.1$	<0.001
Surface roughness (μm)	$1.58^{a} \pm 0.474$	$1.72^{a} \pm 0.183$	$2.03^{a,b} \pm 0.238$	$2.36^{b} \pm 0.273$	0.002
Flexural strength (MPa)	$34.06^{b} \pm 5.89$	$36.58^{b,c} \pm 2.16$	$46.75^{c} \pm 15.34$	$16.15^{a} \pm 2.75$	<0.001

X mean, SD standard deviation. Different superscript lowercase letters in the same raw indicate significant difference among groups. *p is significant at 5% level.

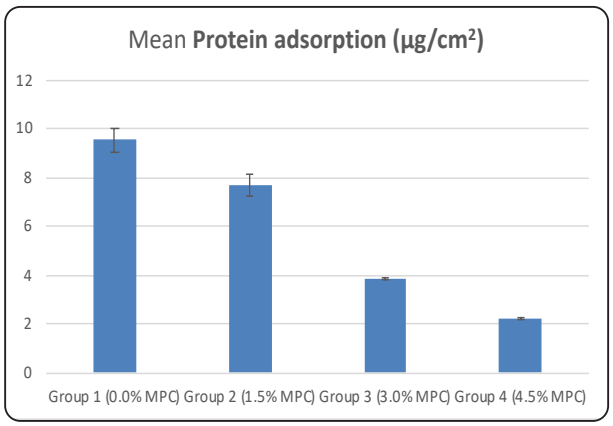

Fig. (5) Bar graph showing the means and standard deviations of protein adsorption (μg/cm2) for the tested groups.

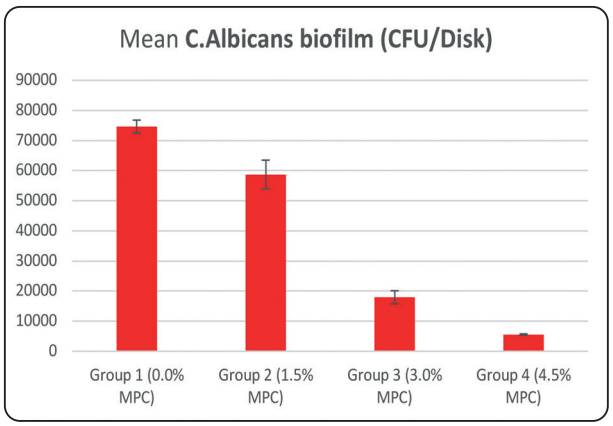

Fig. (6) Bar graph demonstrating the means and standard deviations of C. albicans biofilm (CFU/Disk) for the tested groups.

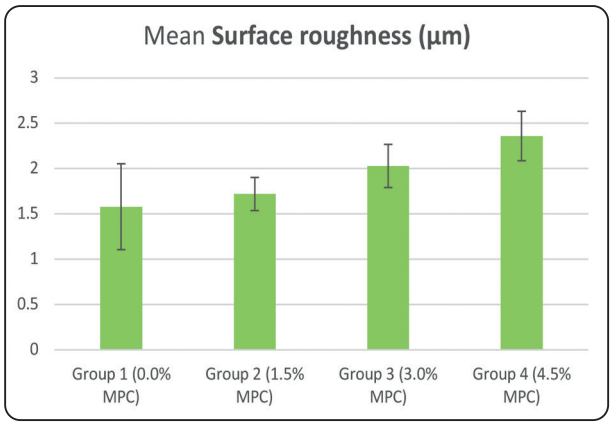

Fig. (7) Bar graph showing the means and standard deviations of surface roughness (μm) for the tested groups.

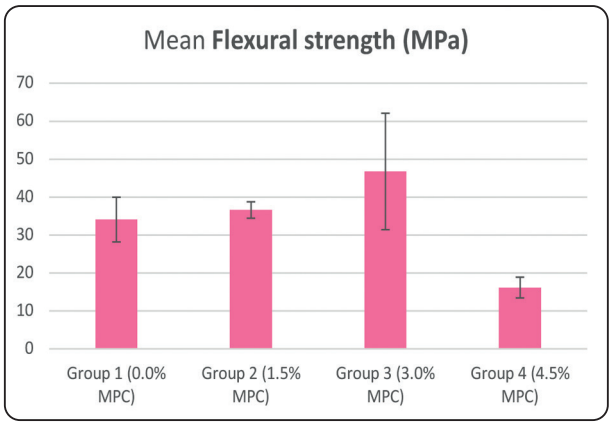

Fig. (8) Bar graph demonstrating the means and standard deviations of flexural strength (MPa) for the tested groups.

DISCUSSION

Removable appliances play a remarkable role in orthodontic treatment. They are considerably utilized either to perform simple tooth movement or as a primary intervention to allow growth modification. Furthermore, they take a considerable share in maintaining the desired occlusion after fixed orthodontic treatment and prohibiting relapse. [20]

Resins containing polymethyl methacrylate are vastly utilized for various purposes such as removable orthodontic appliances. The main problem concerning these appliances is plaque aggregation, as the rough and porous surface is considered a perfect media for microorganisms particularly fungi. [21]

Biofilm Production resulting from growth of fungi in relation to a surface is a highly popular phenomenon. Biofilms represent microbial organizations coated with a matrix of compound extracellular polymeric material.[22]

Rough PMMA surface collect further plaque and proteins, enhancing fungal aggregation and propagation as it comprises irregularities and voids which augments the retention of candida albicans.[8]

Fungi penetrate the acrylic resin micropores, decreasing its mechanical charateristics via the enzymatic action or formation of volatile metabolites.[23] Subsequently, it would be beneficial to develop a novel polymethyl methacrylate resin with convenient mechanical characteristics that could prohibit protein adsorption and fungal adherence.

The protein repellent MPC is a hydrophilic and biocompatible polymer. It has been proven to possess superior capability to repel proteins and hinder microbial adherence. [24] The mechanism of protein repellency of MPC is due to its phospholipid composition that includes a hydrophilic head and hydrophobic tails. The high quantity of free water surrounding the hydrated 2-methacryloyloxyethyl phosphorylcholine is supposed to release protein & prohibit its adsorption. [25] Thus, the goal of the current work was to explore the impact of integrating three concentrations of MPC into self-cure orthodontic acrylic resin on the protein adsorption, biofilm film formation, surface roughness and flexural strength.

In the present study, the first hypothesis was accepted because a significant decrease in protein adsorption was detected coinciding with the increase in MPC concentration. Furthermore, there was a significant reduction in candida albicans biofilm colony forming unit parallel to the increase in MPC polymer ratio. Therefore, the second hypothesis was also accepted. These results are in consistency with

the findings of Choi et al. (2020)[26] who revealed significant reduction in protein adsorption and microbial adhesion after incorporation of MPC into an adhesive system. Similarily, Bajunaid et al., 2021[8] reported that adding 4.5% MPC into high impact denture acrylic resin had decreased the candida albicans biofilm colony forming unit by one order of magnitude.

Unlike Maaly et al 2021[27] who suggested that surface roughness was decreased for for MPC coated denture bases, our study showed that MPC incorporation at the highest weight percentage of 4.5% greatly increased the roughness magnitude, while the lower concentrations did not compromise the surface roughness. Therefore, the third null hypothesis was partially rejected. This is in accordance with the results of Lee, 2013[28] who found a significant decrease in surface roughness with increased MPC concentration in composite resin.

The flexure strength of resin is of paramount importance as it is a measure of its impedance to fracture and hardness.[29] In the present work, incorporating 3 wt% of MPC into self-cure orthodontic resin ameliorate the flexure strength, while adding its highest weight percentage of 4.5% caused deterioration of the flexural strength. Therefore, the fourth null hypothesis was also partially rejected. These findings agree with those of Cao et al[30] who declared that addition of 3% MPC into acrylic resin achieved potent protein repellency without jeopardizing the mechanical properties. Also, a recent study revealed that bioactive polymethyl methacrylate resin including 3 % MPC showed excellent flexural strength after six months of water aging. [23]

CONCLUSION

Incorporation of 3 wt% MPC into removable acrylic orthodontic appliances could accomplish protein repellent and antifungal properties without jeoparadizing the surface roughness or inducing adverse effects on the flexural strength.

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