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ASSESSMENT OF ENAMEL DEMINERALIZATION RESISTANCE AND SHEAR PEEL BOND STRENGTH OF PROTEIN REPELLENT ORTHODONTIC GLASS IONOMER CEMENT

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ABSTRACT

Objective: The purpose of this work was to create a novel orthodontic glass ionomer cement (GIC) modified with a protein repellent substance to resist enamel demineralization without compromising the bond strength.

Materials and Methods. 2-Methacryloyloxyethyl phosphorylcholine (MPC) was incorporated into the GIC at 0, 1.5, 3, 5 wt. %. Teeth were bonded to stainless steel bands then immersed in demineralizing media. Bands were removed then teeth were sectioned. Enamel surface morphology, calcium and phosphorous wt. % were evaluated utilizing the scanning electron microscopy combined with energy dispersive X-ray spectroscopy. Shear peel bond strength (SPBS) was assessed using a universal testing machine. The adhesive remnant index (ARI) was employed. The data were statistically analyzed by one-way ANOVA. Chi square test was used to compare between groups for ARI.

Results: Ca and P wt. % were significantly increased with increasing MPC ratio. Pores and cracks on enamel surface were significantly decreased with 1.5, 3 % MPC, while 5 % MPC showed glossy enamel surface with absence of cracks and porosity. SPBS of 1.5, 3 % MPC showed non-significant change compared to the control group, while there was significant decrease for 5 % MPC modified GIC.

Conclusions: Adding 3 % MPC into GIC used for cementation of orthodontic band is efficient for the resistance of enamel demineralization without any significant influence on the shear peel bond strength.

KEYWORDS: Enamel demineralization, Shear-peel bond strength, Protein repellent, Orthodontic cement.

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INTRODUCTION

Orthodontic treatment becomes more prevalent as it can decrease the incidence of dental diseases, ameliorate the facial esthetics, fix malocclusion, promote the oral tasks and minify the psychosocial issues related to bad facial and dental appearance. Fixed orthodontic appliances are applied for at least one year in the oral cavity, which may encourage the plaque accumulation in spite of proper oral hygiene.[1]

White spot lesions (WSLs) represent the usually detected adverse effects resulting from microbial aggregation around fixed attachments in orthodontics as they disrupt the oral hygiene measures and normal remineralization.[2] Salivary proteins can be adsorbed on the surface of enamel close to bands and constitute the salivary pellicle that act as base for bacterial engagement and is crucial for biofilm creation.[3] The mature biofilm uptakes carbohydrates and form organic acids that cause enamel demineralization which is manifested as white spot lesions.[4]

The demineralization could become remarkable as early as four weeks after treatment commencement and the prevalence of white spot lesions in orthodontics is as high as 60.9%. [5] Glass Ionomer Cement (GIC) has been detected with better remineralizing efficacy than resin composite adhesives as a result of fluoride releasing characteristic. Although, very restricted evidence assured that glass ionomer cement is valuable in decreasing the appearance of white spot lesions compared to resin composite. The fluoride release level from GIC could not have efficient antimicrobial concentration and it is quickly reduced by time. Thus, glass ionomer cement is not able to reduce the white spot lesion incidence through inhibiting biofilm creation and combating microbes.[6]

During orthodontic mechanotherapy, it is hard to accomplish oral hygiene measures on the banded teeth. Thus, more microorganisms are aggregated, causing a decrease in pH that direct the demineralization-remineralization balance toward mineral loss (demineralization).[7]

Subsequently, novel bioactive orthodontic cement that can cause neutralization of the acidic medium, alleviation of demineralization of enamel close to the bands, and facilitation of the remineralization influence would be highly favourable.[1] As cement type influences the amount of enamel demineralization after post-orthodontic debanding therefore, it is crucial to evaluate the enamel demineralization potential of orthodontic cements.[8]

2-Methacryloyloxyethyl phosphorylcholine (MPC) is a methacrylate that has in its side chain a phospholipid polar group and is capable of repelling protein adsorption. MPC was added to glass ionomer cement producing protein-repellent action and preventing biofilm and bacterial accumulation.[9] To date limited dental studies assessed the effect of this MPC modification on preventing the enamel demineralization or affecting the bond strength of GIC to tooth surface. So the current study was done to formulate a novel orthodontic cement with demineralization preventive effect without jeopardizing the bond strength. The null hypothesis was that the incorporation of MPC into GIC would neither change the enamel demineralization resistance nor affect the shear peel bond strength to tooth

MATERIALS AND METHODS

Sample size analysis

Sample size determined based on an earlier study results[10], which revealed that the minimum acceptable sample size for each group was 20 teeth, at the power of 80%, the type I error probability was 0.05, and the mean ± standard deviation of calcium weight in enamel for group I was 47.91 ± 5.43. The estimated mean difference with the other group was 5. To do the t test, P.S. power 3.1.6 was used.

Study design

The research was conducted experimentally in vitro. Steps of the present study were accepted by the Institutional Review Board, Faculty of Medicine, Zagazig University with ethical number 11222-24-10-2023. The assessed materials were, GIC (Medicem, Promedica Dental Material GmbH, Domagkstrasse, Neumuenste, Germany) and MPC (Sigma Aldrich, St. Louis, MO, USA traditional). GIC is a traditional GIC that is based on powder and liquid and is chemically cured. It first forms through an Acid–base interaction, which is then followed by a cross-linking process. At different weight percentages (1.5, 3.0, 5.0, wt. %), MPC was added into GIC powder while GIC powder without MPC was utilized as a control. For ten minutes, a mortar and pestle were used to mix. A 0.0001 g precision balance (TS4000, Ohaus, Pine Brook, NJ, USA) was employed. [11]

One hundred and sixty extracted human upper premolars teeth for the purposes of periodontal reasons or orthodontic purposes were collected from Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Mansoura University. The premolars had no surface irregularities or restorations, and they were free of cavities, cracks and white spot lesions. After being thoroughly cleaned of any blood or debris with a rubber cup, teeth were polished using fluoride-free pumice paste. They were kept in a daily-changing solution of double-deionized water that was supersaturated with 1% thymol until they were used.[8] The study used pre-formed orthodontic unwelded stainless steel premolar bands from Dentaurum in Ispringen, Germany. Bands were adjusted on each premolar in accordance with the prescribed size and have inner surfaces that have been roughened to provide safe retention and ideal bonding. To evaluate enamel demineralization resistance and SPBS, teeth were distributed into four groups of twenty each according to the modified cement, as following:

Group I: unmodified GIC powder (control).

Group II: GIC powder + 1.5 % MPC.

Group III: GIC powder + 3 % MPC.

Group IV: GIC powder + 5 % MPC.

1. Evaluation of enamel demineralization resistance

Band cementation

Using band pinching pliers, bands were firmly pressed around the teeth. A band seater was then used to fit and seat the bands with good marginal adaptation. The manufacturer’s directions were followed while cementing bands to teeth. Bench setting of cements was permitted at constant room temperature. After band placement, dry cotton wool rolls were used to remove any leftover cement. [8]

Demineralization process

Teeth were submerged in a synthetic demineralizing solution with 4.4 pH, consisted of $CaCl_2$, NH_2PO_4, and acetic acid. Each group was immersed in a glass container containing the demineralized solution for four weeks. Every week, the solution was replaced to prevent possible fluoride buildup .[12]

Sectioning of the teeth

Upon removal of teeth from the solution, the bands were detached using band-removing pliers and teeth were cleaned under running water. The roots of every tooth were attached vertically in self-curing acrylic blocks (Acrostone acrylic resin, Dentsply Sirona, York, PA, USA) for sectioning. The teeth were sectioned mesiodistally through the middle of the exposed enamel using a slow-speed diamond saw (IsoMet 1000, Buehler) that was water-cooled. Then, every section was taken out of the remaining fixation block components and ultrasonically cleaned.[13]

Elemental analysis

Sections were placed in a dehydrator (Bel-Art Inc, Wayne, USA) containing silica for 24 hours.

The calcium and phosphorus weight percentages in various sections (demineralized enamel) were measured using the SEM-EDX (TESCAN VEGA 3, Czech Republic) with 20 kV of voltage and 500× magnification.[14]

Surface analysis

SEM was used to examine the specimens' enamel surfaces. Specimens were attached to aluminum stubs and coated with gold (Au) using a Quorum methods Ltd., sputter coater (Q150t, England) prior to analysis. For every group, microphotographs were captured at 100× magnification.[14]

2. Shear Peel Bond Strength

Band preparation and cementation

After the teeth were completely dry, the crowns were prepared for banding. The roots of the teeth were vertically embedded in cylindrical rings that were filled with self-curing acrylic resin. Two weldable sheaths (Dentaurum, Ispringen, Germany) were spot welded and soldered to the midbuccal and midpalatal band surfaces, respectively, for each of the chosen bands, parallel to the band's occlusal plane. For each band, two U shaped 0.036" stainless steel wire with the same length, engaged the welded sheath on both buccal and palatal sides, the four ends of the 0.036" wire were soldered together at their ends in straight form to be mounted to the head of the testing machine, Figure 1.

The cementation process was started following the manufacturer's recommendations. The cement was loaded into each stainless steel orthodontic bands and seated on the tooth with hand pressure then with band seater. Margins were adapted by band pusher. Specimens left for setting for 10 min, excess cement was removed with dry cotton wool rolls. Then stored in a saline at 370˚C and 100% humidity for 24 hours in an incubator to stimulate ideal oral environment.[15]

Shear peel bond strength evaluation

Each mounted sample was attached in a universal testing device (Model LRX-plus; Lloyd Instruments Ltd., Fareham, UK) set to a crosshead speed of 1 mm/minute in order to ascertain the SPBS. Until the band was completely withdrawn from the teeth, testing was carried out. Newtons were used to represent the maximal force required for entirely removing the band from the teeth. After cleaning and cutting of the band with band cutting scissors, it was put down flat to measure its length and width with a millimeter caliper to the closest tenth of a millimeter, allowing the area to be calculated in millimeters. Through dividing the load reading by the band surface area (1 MPa = 1 N/mm^2), SPBS was computed.[15]

Adhesive Remnant Index

Following de-bonding, the specimens were examined under a magnifying lens (x10 times magnification) to identify the remnant of cement location, ARI Scoring was as follows: 0 represents no cement persists on the tooth surface; 1 represents area covered by cement is less than half the crown; and if more than half of the tooth surface is 2; finally, when all of the tooth surface beneath the band is enclosed by cement, the score is 3.[15]

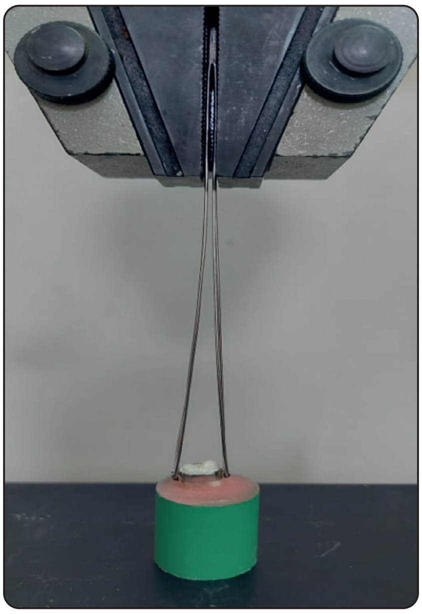

Fig. (1): Mounted sample on the testing machine.

Statistical analysis

Windows Excel, Graph Pad Prism, and SPSS 16® (Statistical Package for Scientific Studies) were used. Shapiro-Wilk and Kolmogorov-Smirnov tests were used to explore the provided data for normality, and the results showed that the data came from a non-parametric distribution. One Way ANOVA test, was the used way to associate groups, and Tukey's Post Hoc test was utilized for multiple comparisons. Meanwhile, the Chi square test was employed to compare groups in the ARI score (qualitative data). A significant threshold of $p \leq 0.05$ was established.

RESULTS

SEM-EDX results

Representative SEM images of an enamel surface cemented with the experimental GICs after demineralization process are shown in Figure 2. Group I showed moderate cracks and fissures over the enamel surface because of enamel demineralization. Obvious porosity and surface irregularities presented in demineralized enamel surface. Minor erosion and porosity were also observed in the vicinity of the band-tooth interface. Additionally, regions of stripped enamel were apparent on the enamel surface, exposing the enamel rods in a vivid manner. The enhanced porosities in the enamel, together with the loss of prismatic structure, were noted (marked with arrow). Group II showed decrease in number and size of cracks and pores due to demineralization resistance, more decrease of pores and cracks in group III. Group IV revealed the melting of enamel rods, resulting in a smooth, glossy enamel surface with well-coalesced enamel rods. The enamel's porous structure was also absent.

Representative EDX images of calcium and phosphorous wt. % for all groups are shown in Figure 3. Comparison between different groups revealed that there was a significant difference among all groups ($P<0.0001$) as presented in Table 1 and Figure 4. Multiple comparisons demonstrated that calcium and phosphorous wt. % increased following increasing MPC ratio. The lowest values were recorded for group I (43.33±2.88), (10.47±0.89) while group IV recorded the highest values (73.48±3.48), (14.96±1.03) with significant differences. The highest Ca\P ratio was for group III (5.94±0.71), group I (4.18±0.52) was significantly the lowest, then group II (4.63±0.4) and group IV (4.94±0.44) with insignificant difference between them.

Shear peel-bond strength results

Mean and standard deviations for SPBS (MPa) of all groups are presented in Table 1 and Figure 5. Comparison among all groups displayed significant difference among them as $P<0.001$, followed by multiple comparisons which revealed that group IV (0.97±0.27) showed significantly lower bond strength, while there was insignificant difference between other groups.

ARI scores among all groups were presented in Table 2 and Figure 6. Comparison between all groups revealed insignificant difference between them as $P=0.06$, in all groups, ARI score 0 was higher than ARI score 1 & 2.

TABLE (1). Mean and standard deviation values of SPBS strength, Calcium &Phosphorous wt% and (Ca/P) ratio for all groups.

Group	Calcium (wt%)	Phosphorous (wt%)	(Ca/P) ratio	SPBS(MPa)
Gr I	43.33 ± 2.88 [a]	10.47 ±0.89[a]	4.18 ± 0.51[a]	1.57±0.44 [a]
Gr II	52.37 ±2.75 [b]	11.37 ±0.92[b]	4.63 ±0.40[b]	1.44 ±0.39[a]
Gr III	64.87 ± 3.02 [c]	11.04 ±1.05[b]	5.93 ± 0.71[c]	1.29 ±0.30[a]
Gr IV	73.48 ±3.48 [d]	14.96 ±1.03 [c]	4.94 ±0.44[b]	0.98 ±0.26[b]
P value	<0.0001*	<0.0001*	<0.0001*	<0.0001*

Significant difference as P<0.05.
Means with different superscript letters were significantly different as P<0.05.
Means with the same superscript letters were insignificantly different as P>0.05.

TABLE (2) Frequency and percentages of different ARI scores among all groups.

Score	Group 1		Group 2		Group 3		Group 4		Chi square test	
	N	%	N	%	N	%	N	%	Chi square	P value
0	12	60.0%	14	70.0%	14	70.0%	19	95.0%		
1	6	30.0%	6	30.0%	6	30.0%	1	5.0%	11.76	0.06
2	2	10.0%	0	0.0%	0	0.0%	0	0.0%		

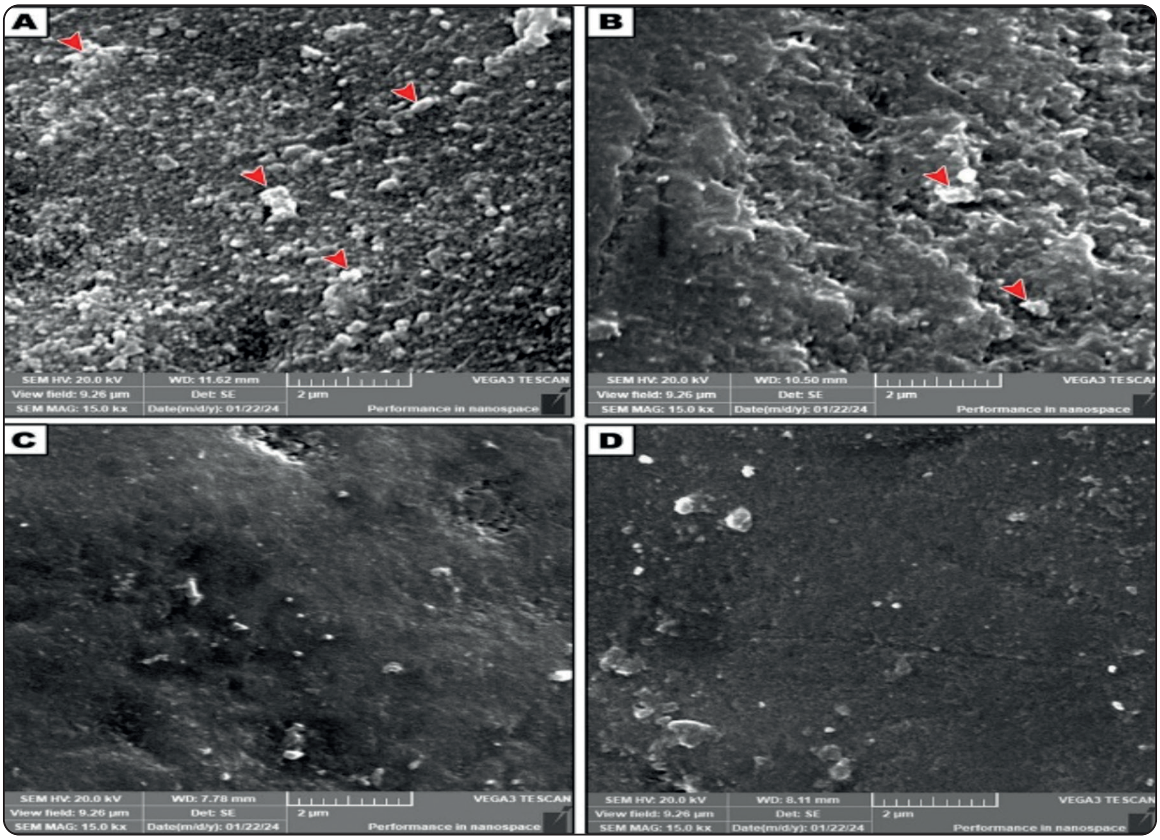

Fig. (2) SE micrographs of demineralized enamel surface a) 0% MPC, b) 1.5% MPC, c) 3% MPC and d) 5% MPC

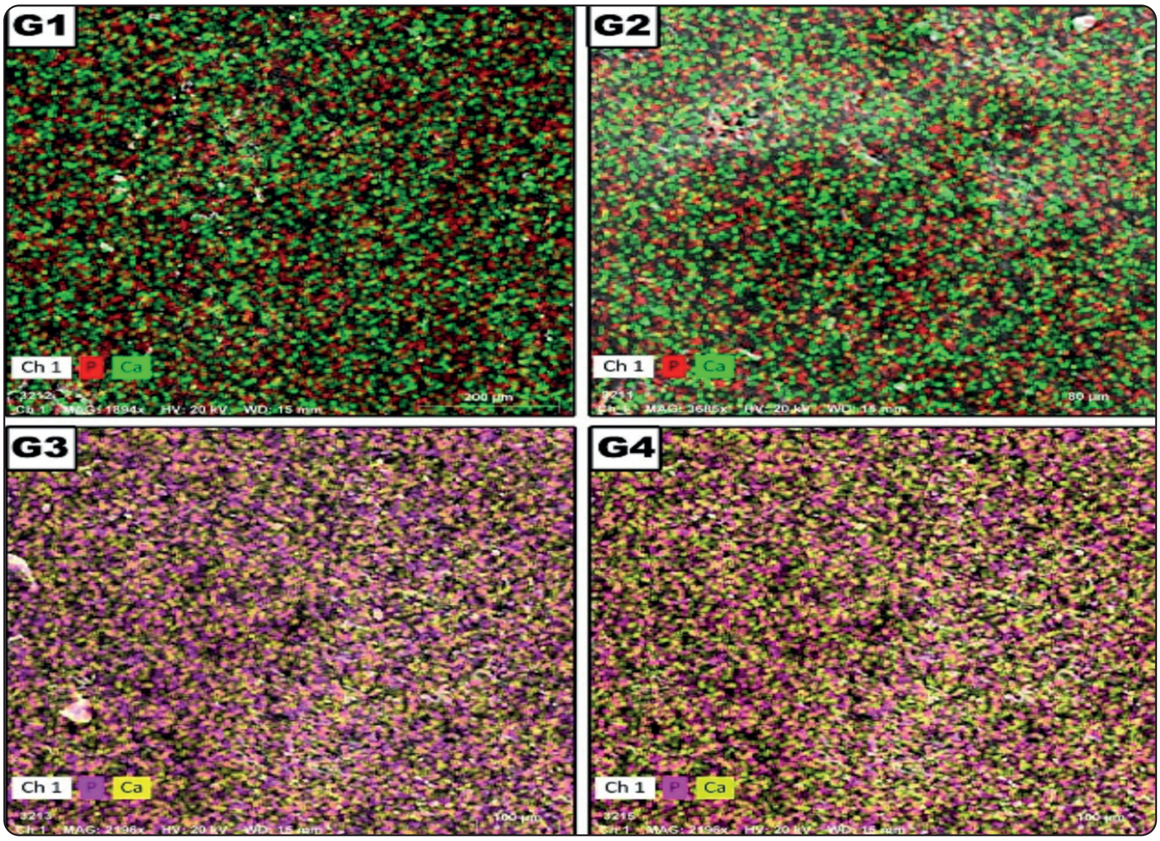

Fig. (3) Representative images of calcium and phosphorous on demineralized enamel G1) 0% MPC, G2) 1.5% MPC, G3) 3% MPC and G4) 5% MPC

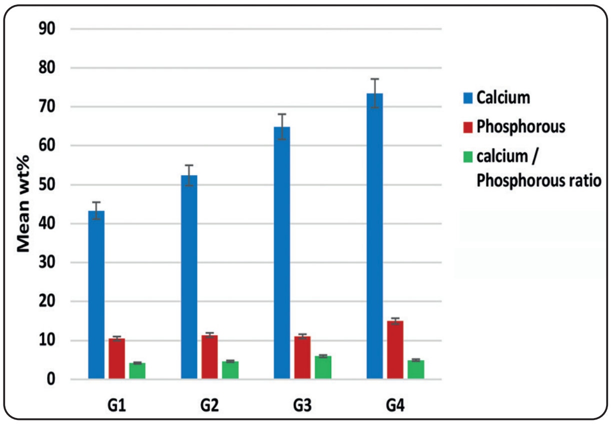

Fig. (4) Bar chart showing minerals contents in all groups

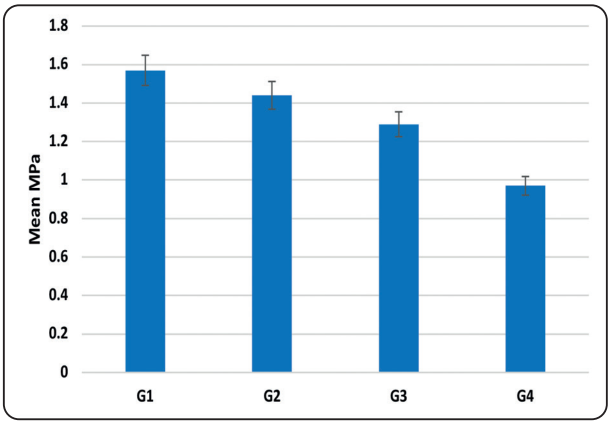

Fig. (5) Bar chart showing bond strength in all groups

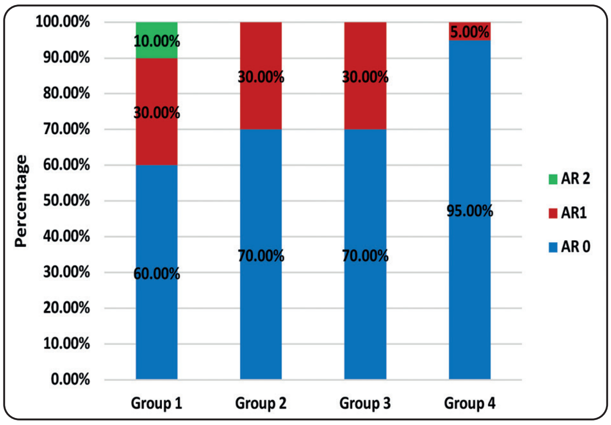

Fig. (6) Bar chart showing different ARI scores among groups.

DISCUSSION

Clinical experience demonstrated that orthodontic banding, with its welded attachment, enhances the susceptibility of enamel decalcification by acting as an accommodation for plaque buildup.[16] Compared to brackets, bands are thought to promote more enamel demineralization.[17] Therefore, to reduce bacterial attachment and biofilm formation at this weak link, it is desired to produce adhesive cements that resist the attraction of salivary proteins. Enamel demineralization brought on by bacterial metabolism that eliminates Ca and P ions from the hydroxyapatite crystals that comprise the enamel rods. This creates a porous structure that permits the entry of water and air.[18]The current study established a novel orthodontic dental cement via incorporation of different ratios of MPC in a conventional GIC. The rationale for this material was to prevent enamel demineralization that occur in the area adjacent to orthodontic bands while maintaining the bond strength.

In this work, the enamel demineralization resistance of different groups was assessed using the SEM-EDX method. It is an analytical method for elemental analysis, both quantitative and qualitative. This method's objective was to ascertain how GICs under study affected the chemical constituents of demineralized enamel. EDX makes it possible to determine how much minerals are in the sample and this data allows for the determination of the Ca/P ratio.[19, 20] The results of our study exhibited a statistically significant increase of the minerals content and Ca/P ratio of the demineralized enamel with the increased concentrations of MPC. Unmodified GIC recorded the lowest Ca and P wt. %, while 5 % MPC recorded the highest values with significant difference. An increase in the Ca/P ratio appeared to cause a covering layer to be deposited over the enamel prisms, decreasing the porosities. Modified GIC with 3 % MPC recorded significant highest ratio while the lowest one was for unmodified powder.

SEM is distinguished by higher energy beam electrons that are scattered to display information indicating subsurface changes in the composition. It was used in this work to provide surface/layer morphological information and to qualitatively estimate enamel demineralization. [21,19] Compared to the control group, reduced cracks and porosities were observed with 1.5 and 3% MPC, while 5% MPC showed smooth enamel surface free from porosities and cracks.

The findings are align with a study[11] which revealed that GIC containing MPC can prevent tooth demineralization by reducing bacterial adherence and acid generation. Zhang et al. discovered that MPC lacks the ability to remineralize, and that in order to more successfully prevent biofilm formation, antimicrobial and remineralization ingredients must be added to dental composites.[22] On the other hand, Lee et al. proposed that increased MPC loading increases mineral content and ion discharge.[23] Park et al. demonstrated that MPC-incorporated orthodontic bonding agents enhanced remineralization effects. [24] Kwon et al. found that the application of light-cured varnish containing 3 % MPC effectively prevented enamel demineralization and also, enhanced apatite precursor formation by assistance with mineralization on MPC-incorporated calcium silicate cement.[25]

The results can be explained by the fact that MPC raises the surrounding media's pH above 5.5, as below that, hydroxyapatite dissolves, removing calcium from the tooth structure and causing tooth demineralization. [26] The ratio of 5%, compared to lesser MPC percentages, demonstrated a quick increase in pH in a previous study.[27] The inclusion of MPC in the form of a monomeric, amine-containing formulation improved the neutralization rate and amplified the pH neutralization of the dental adhesive.[28] Therefore, in addition to providing biofilm resistance, MPC also raises pH, which helps to neutralize the environment surrounding the dental materials.

MPC exhibits both cationic and anionic groups, yet the material's total charge is neutral.[29] Phospholipid polar groups found in the side chain of MPC, a type of lipid with hydrophilic heads and hydrophobic tails. It forms a bilayer when they are submerged in water, with the polar heads facing outward and interacting with the water. Non-polar tails facing the interior region of the bilayer so creating extremely hydrophilic surface.[30] Free water diluted the acidic media, therefore, hydrogen ions concentration decreases and the pH increases toward the neutrality. Earlier studies [31, 32] demonstrated that the hydrophilic nature of MPC repels proteins and lessens the adherence of bacteria as MPC has no bactericidal effect.

Bands are more likely to loosen and fail because they are subjected to tensile and shear pressures during mastication.[15] Shear bond strength is evaluated in orthodontics due to its repeatability and combining shear and peel forces.[33] Bond strength should be maintained high enough to prevent appliance failure during treatment, but low enough to permit debonding without enamel damage.[34] Results of this study revealed no significant differences of SPBS between the control, 1.5% and 3% MPC specimens, while 5% MPC recorded significant reduction of the bond strength. The results are in agree with a previous study[24] claimed that the SBS value was noticeably low for 5% MPC, so adding too much MPC can deteriorate the material's mechanical qualities. Other study [35], found that bond strength of the bonding agent containing 7.5% MPC was comparable to the control. The fact that MPC and the adhesive were miscible in each other without agglomerations or clusters formation explains this outcome. It was found[36] that higher ratio was likely to cause MPC to gel, which would decrease the mechanical characteristics when the particles clumped together. The agglomerated compounds could behave as matrix for stress concentration spots, which would be unfavorable to the mechanical properties of the material, thus, using MPC at low concentrations is indicated.

The amount of cement remaining on the tooth following debonding was assessed using the Adhesive Remnant Index.[37] Short time is required to remove cement from the tooth surface after debonding with low ARI score. This indicated that the bond failure has happened at the enamel-adhesive contact and very little cement was left on the enamel surface.[33] Bond strength data did not always meet with the findings of ARI. [38] Results of present study revealed that the statistically significant differences in bond strength values did not correspond with ARI rank. There were no obvious differences in the ARI ratings between the groups at the failed locations. Since all groups had ARI scores of 0, there is no cement left on the tooth.

Previous studies[11,32,34] found that 3% MPC has high antibacterial effect, achieving durable repelling of protein while maintain the physical and mechanical properties of the material. This is in accordance with our study which concluded that 3 % MPC is the selected ratio for modification of orthodontic GIC due to its ability to resist enamel demineralization without compromising the retentive strength.

CONCLUSIONS

1. All modified orthodontic GIC with MPC exhibited significant increase in calcium and phosphorous wt. %, also there was significant reduction of cracks and porosity compared to unmodified one.
2. Modified 5% MPC cement exhibited smooth enamel surface free from cracks or porosity and recorded significant increase in the minerals wt. %.
3. Modified 1.5, 3 % MPC cement recorded non-significant change in SPBS, while 5 % MPC recorded significant reduction values.
4. Based on our results, orthodontic GIC modified with 3 % MPC is very promising formulation that exhibited high resistance to enamel demineralization with an acceptable retentive strength to the tooth.

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