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Evaluation of Antibacterial Activity and Compressive Strength of Protein Repellent Nanostructured Orthodontic Modified Glass Ionomer Cement

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ABSTRACT

Objectives: To assess both antibacterial and compressive strength values of modified glass ionomer cement (GIC) with 2-methacryloyloxyethyl phosphoryl choline (MPC) and titanium dioxide nanoparticles (TiO_2 NP). ***Methods:*** Three groups were evaluated in the study, unmodified glass ionomer cement (UMGIC) as a control group, (GIC) + 3wt % (TiO_2 NP), and (GIC) + 3wt % (TiO_2NP) +3wt % (MPC)**.** Thirty-disc shaped specimens (n=10) were incubated with S. mutans suspension, by agar plate diffusion test, for antibacterial activity assessment. Zones of bacterial growth inhibition were recorded in millimetres (mm). For compressive strength testing, the prepared specimens were transferred to a universal testing machine. Statistical evaluation was performed with one-way ANOVA test. ***Results:*** (GIC) + 3wt % (TiO_2NP) +3wt % (MPC) recorded the highest diameters of growth inhibition zone against streptococcus strains, while lowest values were for unmodified GIC**.** ANOVA indicated statistically significant difference between the different groups. (GIC) + 3wt% TiO_2 NP recorded the highest compressive strength value while unmodified GIC recorded the lowest values with significant difference among different groups. ***Conclusion:*** Combination of the double agents, TiO_2 NP and MPC, protein repelling polymer, into GIC attained a higher antibacterial effect, without jeopardizing its mechanical properties.

Keywords: Antibacterial, Glass ionomer cements, TiO2 NP, MPC, Compressive strength.

Introduction

Dental caries is regarded as the most widespread infectious oral disease (Ferracane, 2011). Oral biofilms produce acids that could demineralize tooth structure causing such caries (Eltahlah *et al.,* 2018). It is a problematic matter for the orthodontic appliances to preserve a good hygiene of dental structure, thus causing growth of biofilm plaque. Glass-ionomer cement is largely applied for orthodontic band cementation due to the claims of its anti-cariogenic property by release of fluoride ions (Olivia *et al.,* 2000).

Despite the ability of GIC for releasing fluoride, the causative factor for GIC failure is the secondary caries. So fluoride-release is not satisfactory to avert bacterial growth creating insufficient antibacterial efficacy of GIC (Xie *et al.,* (2011). Merging the fluoride with antimicrobial components were tried in many studies since fluoride only is inadequate for inhibition of decay (Bob ten Cate, 2009). Consequently, the addition of antibacterial components into GIC might demonstrate a practical assistance in caries prevention. Numerous trials have been done to develop antibacterial properties of GICs, such as, addition of chlorhexidine gluconate and quaternary ammonium salt to GICs (Marti *et al.,* 2014). Unfortunately, this addition of bactericides created bad effects on the mechanical capability of GICs. Therefore, it is important to select antibacterial agents and to detect the precise amount for GICs modification.

Nanotechnology has been used in dentistry to enhance mechanical and antibacterial properties of dental materials (Ozak and Ozkan, 2013). Titanium dioxide nanoparticles (TiO_2 NP) are frequently applied for dental and medical fields (McIntyre, 2012). Previous studies showed that TiO_2 nano-particles exhibited a significant antimicrobial effects (Kasraei *et al.,* 2014). Bacteria are less likely to develop resistance against it, so the use of TiO_2 nanoparticles has a good potential for preventing white spot formation.

Extra trials were done to produce materials, that retain a capability of repelling bacteria, by covering its surface by an extremely hydrophilic film (Müller *et al.,* 2009). The most prevalent

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polymer achieved both biocompatibility and hydrophilicity is, 2-methacryloyloxyethyl phosphorylcholine (MPC), it has a polar phospholipid group, attached to lateral chain in a methacrylate (Ishihara *et al.,* 1990). MPC polymers are recognized to lessen adsorption of protein and bonding of bacteria to surfaces, so it is used for coating of the surfaces (Ishihara *et al.,* 1998).

In recent studies, incorporation of MPC to dental composites and bonding agents creating a new protein-repellent dental materials (Zhang *et al.,* 2014, 2015). It was also added into a RMGIC (Zhang *et al.,* 2016). However, that combination didn't increase the antibacterial property of the material (Zhang *et al.,* 2016). It would be valuable to add MPC with an addition of TiO_2 NPs to keep protein-repulsion and bacterial resistance capabilities. Consequently, this research aimed to create a dually modified orthodontic GIC, glass powder with TiO_2NP and MPC and to evaluate this modification on the antibacterial activity and compressive strength.

Materials and Methods

Modification of Glass-Ionomer Cement.

Materials that evaluated in our study are shown in table 1. Modified powder was prepared by mixing of the different components in a vortex for one minute, the following groups were evaluated in the study:

Group1: Unmodified glass ionomer cement (UMGIC) as a controll group.
Group2: (GIC) + 3wt% (TiO_2 NP).
Group3: (GIC) + 3wt% (TiO_2 NP) + 3wt% of (MPC).

Table 1: The used materials in the study.

Materials	Composition	Manufacturer
CG Fuji Ortho	Alumino-silicate glass, polyacrylic acid, Hydroxy-ethyl-methacrylate, Tri-ethylene glycol methacrylate.	GC America Inc, Tokyo, Japan
Titanium Dioxide	Nano-powder of Titanium Dioxide with anatase phase, average size 21 mm, 99.5%.	Sigma Aldrich, St. Louis, MO, USA
MPC	2-methacryloyloxyethyl phosphorylcholine, contains ≤100 ppm MEHQ as inhibitor, 97%.	

Antibacterial test

Mixing of powder and liquid was done according to manufacturer's instructions. It was accomplished using a plastic spatula and a glass slab. Preparation of specimens was done using a Teflon mold (10 mm diameter, 2mm thickness). The mold was hand pressed between two microscopic glass slabs, the glass ionomer was allowed to set at room temperature for 15 min then it was hard-pressed by finger to remove the specimens. A scalpel was used to remove gently excess material. Streptococcus mutans strain type (ATCC 25175) was cultured, at 37°C, in brain heart infusion broth (BHI) (Oxoid, England) overnight. The turbidity of suspension was attuned to the turbidity standard, McFarland 0.5. Inoculum was spread all over BHI agar plate. Instantly, GIC discs were placed over the agar, inoculated with the inoculum and after 24 hours of incubation at 37°C, the diameters of halos of bacterial growth inhibition were measured with electronic digital caliper (Iwanson, Martin, Germany).

Compressive Strength Test

A split Teflon mold (8 mm height, 4 mm diameter) was used to formulate cylindrical shaped specimens. A total number of thirty specimens were fabricated (n = 10) for the three tested groups:, they were transferred to a universal testing machine (LLOYED instruments, LR 5K, England), and subjected to testing at 0.5 mm/min, crosshead speed, then forces at which the material was broken, were recorded. Compressive strength = P/A, calculated in Mega Pascal, where P is the load of failure estimated in newton and A is the cross-sectional area estimated in mm^2.

Statistical Analysis

Collected values were statistically studied by the software, Version 18, SPSS Inc., Chicago, USA. ANOVA, One-way Analysis of variance, and Post hoc Tukey test were performed to determine variances among the groups. Statistically significance difference results when *P*-value is less than 0.05.

Results

Dually modified GIC with 3wt% TiO_2 NP and 3wt% MPC recorded the highest diameters of bacterial growth inhibition zone for streptococcus bacteria (Table 2). (UM GIC), recorded the lowest bacterial growth inhibition zone, possibly producing a comparatively low antibacterial influence. An assessment using ANOVA, displayed a statistically significant difference, ($p < 0.05$) in the value of diameters of inhibition zone against S. mutans, between the three groups. Post hoc Tukey test indicated no significant difference in the diameter values of inhibition zone against S. mutans among GIC + 3wt% TiO_2 NP and GIC + 3wt% TiO_2 NP+ 3wt %MPC.

Table 2: Mean diameter of the inhibit halo (mm).

Group	**Mean** ±SD	P Value
G1: Fuji ortho (GIC)	8.40±0.69	
G2: GIC+3wt % TiO_2 NP	12.23±1.03[a]	P<0.05
G3: GIC+3wt % TiO_2 NP+ 3wt % MPC	13.03±0.97 [a]	

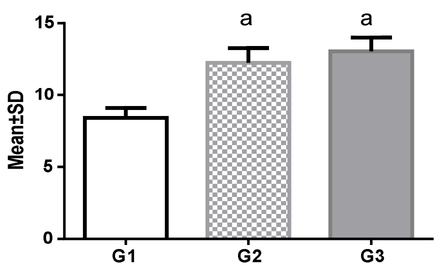

Fig. 1: Effect of GIC and its modifications against S. mutans.

Values of mean and standard deviation of compressive strength for various materials are tabulated in Table 3. ANOVA indicted statistically significant difference ($p<0.05$) of compressive strength values among the various groups. GIC+3wt% TiO_2 NP recorded the highest compressive strength value (90.21±4.11), followed by GIC + 3wt% TiO_2 NP+ 3wt %MPC (86.07±6.48), without significant difference. UMGIC had the lowest compressive strength (70.38±7.18) that is statistically different from that of the other two materials.

Table 3: Compressive strength values (MPa).

Group	Mean ±SD	P Value
G1: Fuji ortho GIC	70.38±7.18	
G2: GIC+3wt% TiO_2 NP	90.21±4.11 [a]	P<0.05
G3: GIC + 3wt% TiO_2 NP+ 3wt %MPC	86.07±6.48 [a]	

Similar superscript letters mean that there was no difference between the materials.

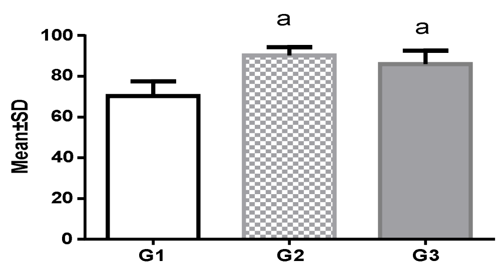

Fig. 2: Comparison between compressive strength values.

Discussion

Several alterations of GICs chemistry were formerly inspected targeting to increase the antibacterial activity without unfavourably affecting its mechanical properties (Moshaverinia *et al.,* 2012). The existing study showed that with the double modification of GIC with TiO_2NP and MPC, a substantial improvement in the antibacterial properties, without compromising the compressive strength, furthermore, with an improvement of the strength compared to unmodified cement.

MPC particles was integrated with GIC at 3 wt%, this ratio was selected following the previous study (Zhang *et al.,* 2016) that showed that, 3wt% of MPC achieved a durable repelling of protein without compromising adhesion to tooth. Adsorbed salivary proteins to the surfaces, offer a favourable condition for adhesion of bacteria, thus starting for biofilm growth (Müller *et al.,* 2009). MPC possess a brilliant protein-repellent capability to impede such bacterial accumulation (Ishihara *et al.,* 1990; Ishihara *et al.,* 1998). As protein attachment results when there is bound water thus, MPC has a protein repelling effect due to its hydrophilic nature and presence of free un bound water in the hydrated polymer part (Ishihara *et al.,* 1990; Ishihara *et al.,* 1998; Katsikogianni and Missirlis, 2004).

On the other hand, the great quantity of water round phosphorylcholine group is the reason for detachment of proteins efficiently, thus, resisting protein adsorption (Ishihara *et al.,* 1998; Goda *et al.,* 2007). It was stated that hydrophobic surfaces are more liable to protein adsorption, in comparison to hydrophilic ones (Cheng *et al.,* 2012.). This was documented by Zhang N et al when they evaluated a novel GIC containing MPC to avoid white spot lesions (Zhang *et al.,* 2016).

The small size of the TiO_2NP (21 nm), giving high surface area, which might enable the diffusion of the TiO_2 particles into the bacterial cell producing intracellular injury (Wetzel *et al.,* 2006). The antibacterial mechanism recommended that species of reactive oxygen (ROS) were detected by TiO_2 NPs, precisely, hydroxyl free radicals and peroxide (Wang *et al.,* 2011). The addition of 3wt % TiO_2 NPs to GIC, exhibited in comparison to unmodified one, improved antibacterial activity against S. mutans. This finding is comparable to the results stated by Elsaka *et al.* (2011)

Test of compressive strength (CS) gives a respectable impression about mechanical capability of dental materials and it is used to inspect breakable materials such as glass ionomer cements (Naasan and Watson, 1998). CS is significant aspect, principally concerning chewing forces. For evaluation of CS, two vertical loads are directed to the specimen in reverse lines, consequently, directing the atomic ingredients toward each other (Wang *et al.,* 2003). Steps of compressive strength testing are not complex. In the present study, GIC modified with 3wt% of TiO_2 NP showed statistically superior value, (90.21±4.11) relative to other cements (Table 3). While the double effect of TiO_2 NP and MPC showed improvement but lower than the single modification (86.07±6.48), without significant difference. Our study agreed with that informed by Elsaka *et al.,* as the ratio of 3wt% of TiO_2 NP improved the mechanical properties OF GIC (Elsaka *et al.,* 2011; Garcia-Contreras *et al.,* 2015).

Enhancement of compressive strength of modified GIC containing 3wt % TiO_2 NP could be ascribed to, the little size of particles of TiO_2, that incorporated into glass powder, also the existence of the NPs can close vacant spaces present among GIC glass particles which is large in size and achieve extra attachment positions for polyacrylic acid (Garcia-Contreras *et al.,* 2015). Regarding the effect of double modification of the cement on the compressive strength, it recorded lower value compared to modified GIC only with TiO_2 NP without significant difference. This is may be due to incompatibility between them or incomplete dissolving or fusion of MPC into GIC powder or with the particles of filler.

Conclusion

1. GIC supplemented with 3wt % TiO_2 NPs and 3wt % MPC is a novel orthodontic adhesive against Streptococcus mutans. However, the ratios which was used in our study may need to be modified for better effects.
2. Extra studies needed to detect the form of interaction between the TiO_2NP and MPC during and after setting of GIC.
3. Setting characteristics, moisture-sensitivity and bonding to tooth, the clinically associated properties of GICs, should be investigated.

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