RESEARCH ARTICLE

Evaluation of Adhesive Bond Strength, and the Sustained Release of Fluoride by Chitosan-infused Resin-modified Glass Ionomer Cement: An *In Vitro* Study

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Abstract

Aim and objective: To evaluate the adhesive bond strength, and sustained release of fluoride in chitosan (CH)-infused RMGIC.

Materials and methods: Twenty caries-free human permanent premolar teeth, extracted for orthodontic purposes, were cleaned and stored in thymol solution. The crown of each tooth was cut into two halves and RMGIC ($n = 10$) and CH-infused RMGIC ($n = 10$) was placed between the two halves of the crown. The tooth was then stored in 10 mL of artificial saliva for a period of 30 days. The fluoride levels of the saliva were checked on the 15th- and the 30th-day using ion chromatography. The adhesive bond strength was checked on the 30th day using a universal testing machine.

Results: This study has shown that the bond strength of RMGIC was not affected by the inclusion of CH in it. Whereas, the sustained fluoride release of CH-modified RMGIC indicated that the fluoride release of CH-RMGIC was 8.47% >RMGIC at the end of 15 days, and, 39.68% >RMGIC at the end of 30 days.

Conclusion: The inclusion of CH in RMGIC does not alter its bond strength, while it does cause a greater release of fluoride.

Clinical significance: In progression with these results, the inclusion of CH in RMGIC could provide desirable properties like mechanical reinforcement effects and catalytic effects on the fluoride release and growth factors.

Keywords: Bond strength, Chitosan, Fluoride release, Glass ionomer cements, Resin-modified GIC.

International Journal of Clinical Pediatric Dentistry (2021): 10.5005/jp-journals-10005-1943

Introduction

Glass ionomer cements (GICs) are the most commonly used restorative material for minimal intervention example, atraumatic restorative treatment (ART) because of their biocompatible nature. However, conventional GICs have some drawbacks including sensitivity to moisture, brittleness, and lower mechanical strength in contrast to other resin-based restorative materials.[1] Accordingly, different alterations have been made to GICs to improve their properties through the addition of different materials such as zirconia,[2] glass fibers,[3] hydroxyapatite,[4] bioactive glass particles,[5] and casein phosphopeptide–amorphous calcium phosphate.[6] Chitosan (CH) is a natural biocompatible linear biopolyaminosaccharide. It has distinct biological characteristics such as it is highly environment friendly, biocompatibility, mucoadhesion, and a wide spectrum of antimicrobial and antibiofilm properties against gram-positive and gram-negative bacteria.[7,8] A previous study[9] has demonstrated the incorporation of different volume percentages of CH in the liquid of traditional glass ionomer and shown a refinement in its physical properties. In this study, we have added CH to the liquid of resin-modified GIC in 10 v/v %, and the adhesive bond strength and the amount of fluoride released by the cement were evaluated.

Materials and Methods

A randomized concurrent parallel experimental design was formulated to assess the fluoride release and adhesive bond strength of CH-modified GC gold-label glass ionomer light-cured universal restorative in, which was conducted in Bharati Vidyapeeth Dental College and Hospital, Pune, India, over a period of 1 month (from 17.07.2018 to 18.08.2018).

The sample size was calculated using Statistical Package for Social Sciences (SPSS ver21.0, IBM Corporation, USA) for MS Windows. Twenty sound human premolars, extracted recently for orthodontic purposes, were collected within a week before conducting the study for the assessment of adhesive bond strength and the fluoride release. Teeth with caries, restorations, fracture lines, or other structural defects were not included in the study, to ensure the sound structure of the enamel and dentin and avoid any errors in the methodology of the study. Ethical approval for the study was obtained from the Ethical and Research Committee of the Institution.

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How to cite this article: Patel A, Dhupar JKMS, Jajoo SS, *et al.* Evaluation of Adhesive Bond Strength, and the Sustained Release of Fluoride by Chitosan-infused Resin-modified Glass Ionomer Cement: An *In Vitro* Study. Int J Clin Pediatr Dent 2021;14(2):254–257.

Source of support: Nil

Conflict of interest: None

Tempering of Glass Ionomer with Chitosan

1.8 mL of glacial acetic acid was increased to 100 mL with distilled water in a 100 mL standard flask to get 0.3 N acetic acid. About 20 mg of CH was weighed separately and mixed with 0.3 N acetic acid. This was then made up to 100 mL with the same acetic acid in a 100 mL standard flask to form 0.2 mg/mL CH solution. Then, 0.1 mL of 0.2 mg/mL of CH solution was mixed with 0.9 mL of RMGIC liquid (GC gold label light-cured universal restorative) to get 10% v/v CH added glass ionomer liquid[10] (Figs 1 and 2).

Sample Preparation

Twenty freshly extracted human premolars were used for this study. Immediately after extraction, the teeth were rinsed under running water and stored in specimen bottles filled with purified filtered water mixed with thymol. These teeth were randomly divided into two groups ($n = 10$).

- Group I—Teeth restored using resin-modified glass ionomer cement.
- Group II—Teeth restored using CH-modified glass ionomer cement.

For group I, the crown of each tooth was sectioned in the middle third using a diamond disk (D: 22 mm, H: 0, 20 mm) mounted on a micromotor and a handpiece under copious water irrigation. The RMGIC powder (GC gold label light-cured universal restorative) of the resin-modified GIC was mixed with the liquid at the mentioned powder/liquid ratio of 3.2/1.0 g for 20–25 seconds. The mixed RMGIC was placed in-between the two halves of the crown and cured for 20 seconds as per the manufacturer's specifications.

Similarly, for group II, the crown of each tooth was sectioned in the middle third using a diamond disk (D: 22 mm, H: 0, 20 mm) mounted on a micromotor and a handpiece under copious water irrigation. The GIC powder (GC gold label light-cured universal restorative) of the resin-modified GIC was mixed with CH-modified liquid at the mentioned powder/liquid ratio of 3.2/1.0 g for 20–25 seconds. The mixed GIC was placed in-between the two halves of the crown and cured for 20 seconds as per the manufacturer's specifications.

Fig. 1: Materials used in the study from left to right; LUX V DTE light cure unit, mixing pad, Agate's spatula, GC gold label light cure glass ionomer cement powder and liquid, sample container, chitosan extract 10 v/v%, wet mouth artificial saliva, metal cutting disk, tooth sectioned in the middle third

The crown and the root of the tooth were then partially embedded in acrylic so that the tooth could be held by the jig of the universal testing machine to check for the adhesive bond strength.

The teeth were then stored at 37°C and 100% humidity for a period of 30 days in 10 mL of artificial saliva (wet mouth). The fluoride release in the saliva was checked on the 15th- and the 30th-day using an ion chromatography test.

All the specimens with the mold made up of acrylic were attached to the jig of the universal testing machine and with a crosshead speed of 1 mm/minute, a shear force was applied at the junction of the enamel cement until fracture occurred and adhesive bond strength was calculated at that point.

Results

Inter-group Comparisons

The distribution of mean ± SD of adhesive bond strength in group I and group II was 26.68 ± 13.13 N and 30.72 ± 11.92 N, respectively. The distribution of mean adhesive bond strength did not differ significantly across the two study groups (p value > 0.05) (Table 1).

Intra-group Comparisons

In group I, the distribution of mean fluoride release at day 30 was significantly higher compared to mean fluoride release at day 15 (p value < 0.001) (Table 2).

In group II, the distribution of mean fluoride release at day 30 is significantly higher compared with mean fluoride release at day 15 (p value < 0.001) (Table 2).

Inter-group Comparisons

The distribution of mean ± SD of fluoride release at day 15 in group I and group II was 18.86 ± 1.91 and 27.33 ± 5.21 mg/L, respectively. The distribution of mean fluoride release at day 15 is significantly higher in group II compared with group I (p value < 0.001).

The distribution of mean ± SD of fluoride release at day 30 in group I and II was 26.94 ± 3.74 and 37.63 ± 6.08 mg/L, respectively. The distribution of mean fluoride release at day 30 is markedly higher in group II in comparison to group I (p value < 0.001).

The distribution of mean % change in fluoride release at day 30 did not differ markedly between the two study groups (p value > 0.05).

Discussion

Glass ionomer cements are commonly used bioactive restorative materials in pediatric dentistry even today. Conventional GICs have some limitations including moisture sensitivity, brittleness, and lower mechanical strength. To improve these properties without significantly affecting their property of chemomechanical adhesion to enamel and dentin various modifications have been made to glass ionomer cements. To overcome its lack of wear resistance and increase its fracture toughness, research efforts were made to reinforce GIC with resin to form resin-modified GIC (self-cured).[10,11] Resin-modified GIC also, decreased the setting time and reduced moisture sensitivity of conventional GICs. They are basically a hybrid of GIC's and composite resin. They contain acid-base and polymerizable components.[12]

Chitosan is a natural linear biopolyaminosaccharide developed by alkaline deacetylation of chitin found organically in the shells of crabs and shrimps.[13,14] It has distinct biological characteristics such as biodegradability, biocompatibility, mucoadhesion, and a

Fig. 2: Universal testing machine used to test the adhesive bond strength of the cement

Table 1: Inter-group comparison of mean adhesive bond strength

	Group I (n = 9)		*Group II (n = 9)*		*Mean difference*	
	Mean	*SD*	*Mean*	*SD*	*95% CI*	*p value (inter-group)*
Bond strength (*N*)	26.68	13.13	30.72	11.92	−4.04 (−16.57 to 8.49)	0.504[NS]

p value by independent sample *t*-test. *p* value < 0.05 is considered to be statistically significant. NS, statistically non-significant

Table 2: Inter-group and intra-group comparison of mean fluoride release

	Group I (n = 9)		*Group II (n = 9)*		*Mean difference*	
Fluoride release (mg/L)	*Mean*	*SD*	*Mean*	*SD*	*95% CI*	*p value (inter-group)*
Day 15	18.86	1.91	27.33	5.21	−8.47 (−12.39 to −4.56)	0.001*
Day 30	26.94	3.74	37.63	6.08	−10.69 (−15.73 to −5.65)	0.001*
% Change at day 30	43.14%		38.72%		4.42 (−10.64 to 19.48)	0.542[NS]
p value (intra-group)						
Day 15 vs day 30	0.001**		0.001**			

p value (inter-group) by independent sample *t*-test, *p* value (intra-group) by paired *t*-test. *p* value < 0.05 is considered to be statistically significant.
**p* value < 0.001, NS, statistically non-significant
***statistically non-significant

wide spectrum of antibacterial and antibiofilm properties against gram-positive and gram-negative bacteria.[7,8] It has also been researched previously in dentistry for its antibacterial effects against *S. mutans*[15,16] with assuring results.

Chitosan in the present study was used together with acetic acid and added to the GIC liquid and the pH value of this liquid was maintained in the acidic range (approximately 1). The solubility of CH in an acidic environment is explained by the protonation of the free amino groups (NH_2) to NH_{3+}.[17] This is because CH is considered to be a strong base as it contains primary amino groups with a pKa value of 6.3. The presence of the amino groups indicates that pH substantially alters the charged state and properties of CH. At low pH, these amines get protonated and become positively charged and that makes CH a water-soluble cationic polyelectrolyte.[1]

It is important that any alteration of RMGIC should not affect the bonding ability to enamel and/dentin. Previous studies have shown an improvement in the bond strength of CH-modified conventional GIC to the enamel, by 84%.[18] A study conducted by Ibrahim et al.[18] showed that incorporation of acidic solutions of CH in the polyacrylic acid, liquid of conventional GIC at v/v ratios of 5–10% did not greatly alter its bonding to dentin surface. Chitosan may be added to RMGIC to enhance its properties; however, the bond strength of CH-modified RMGIC has not been evaluated in any previous studies. Hence, the first aim of this study was to evaluate the adhesive bond strength of RMGIC modified with CH. This study has shown that the bond strength of RMGIC remains unaffected by the addition of CH to it. Chitosan chains have many hydroxyl groups and acetamide groups which can bind to hydroxyl groups of the GIC particles and the carboxyl groups of polyacrylic acid (PAA) by hydrogen bonding. The interfacial tension among the GIC components might be reduced by the network formed by the CH and PAA around the inorganic GIC particles and this might improve or sustain its mechanical performance.[19]

The other aim of this study was to evaluate the fluoride release of CH-modified RMGIC. In this study, we have compared the sustained fluoride release of CH-modified RMGIC at an interval of 15 and 30 days, which has indicated that the fluoride release of CH-modified RMGIC is 8.47% > RMGIC at the end of 15 days, and, 39.68% > RMGIC at the end of 30 days. Also, Petri et al.[16] demonstrated that the amount of fluoride released from CH-modified GIC was much higher than pure GIC and increased as a function of time from 2 to 21 hours to a period of 1 month. Fluoride in the GIC matrix is transported in the form of metal cationic complexes such as aluminum fluoride (AlF2) and calcium fluoride (CaF). Hence, as more calcium and aluminum polyacrylate complexes are formed during the setting reaction, more fluoride ion is released.[9] The mixing of nanochitosan with GIC has a catalytic effect on fluoride release, hence resulting in rapid diffusion of fluoride through the GIC matrix.[9] This rise in fluoride release may help to prevent secondary caries and better remineralization potential of conventional GIC and RMGIC.

Conclusion and Clinical Significance

It can be stated that the results of the current study suggest that modification of RMGIC with CH could provide some clinical significance in dentistry owing to its mechanical reinforcement effects and catalytic effect on the fluoride release and growth factors. However, *in vivo* clinical trials should be conducted involving CH-modified RMGIC for it to have a significant role in the field of dentistry in the coming years.

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