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To study the effects of various agents on the shear bond strength of resin modified glass ionomer cement: In-vitro study

[1]Noushad M C, [1]Professor and HOD Department of Conservative dentistry and Endodontics Kannur dental college, Kerala University of Health Science, Kerala, India

[2]Anish Sebastian, Professor Department of Conservative dentistry and Endodontics Kannur dental college, Kerala University of Health Science, Kerala, India.

[3]Nisha Ann John, Post graduate student Department of Conservative dentistry and Endodontics Kannur dental college, Kerala University of Health Science, Kerala, India.

[4]Nithin A K,Post graduate student Department of Conservative dentistry and Endodontics Kannur dental college, Kerala University of Health Science, Kerala, India

Corresponding Author: Nisha Ann John , Post graduate student Department of Conservative dentistry and Endodontics Kannur dental college, Kerala University of Health Science, Kerala, India.

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Abstract

Objective: To study the influence of different agents on the immediate shear bond strength of resin modified glass ionomer cement (RMGIC).

Materials and methods: Fifty noncarious extracted premolars were flattened on the occlusal surface to expose dentin. The specimens were divided into 5 groups: control, grape seed extract, chlorhexidine, aloe vera extract and guava leaf extract groups (n = 50). In the experiment groups, grape seed extract, chlorhexidine, aloe vera extract and guava leaf extract were applied for 1 min, respectively. Resin-modified glass ionomer was bonded, and the specimens were tested for shear bond strength (SBS) after 24 hours.

Statistical analysis: Data were analyzed with SPSS 22.0 (SPSS Inc, Chicago, IL, USA) and to find out the difference between and within the groups was done using one way ANOVA and tukeys hsd post hoc test.

Results: After 24 hours, the bond strength of chlorhexidine and guava leaf extract groups were significantly lower than that of the control. Whereas the bond strength of grape seed extract group was similar to that of control. Highest shear bond strength values were recorded with the aloe vera extract group tooth specimens.

Conclusion: Within the limits of this study application of aloe vera extract before bonding of resin-modified glass

ionomer to dentin significantly improved the shear bond strength of RMGIC- dentin bonds.

Keywords: Matrix Metalloproteinase, Resin Modified Glass Ionomer, Shear Bond Strength, Analysis Of Variance.

Introduction

Resin modified glass ionomer cement (RMGIC) was introduced to the dental profession in 1991 [1]. They contain the same essential components as conventional glass-ionomers but also include a monomer component and associated initiator system. The monomer is typically 2-hydroxyethyl methacrylate (HEMA), and the initiator is camphorquinone [1]. Resin-modified glass-ionomers set by the twin processes of neutralization (acid-base reaction) and addition polymerization and the resulting material has a complicated structure based on the combined products of these two reactions [2]. The liquid component of RMGIC which contains Polyacrylic acid can be used as a dentin conditioner before bonding with RMGIC. This conditioning step removes the smear layer and exposes the collagen fibrils up to approximately 0.5–1 µm in-depth and a hybrid like layer results from the penetration of resin monomers into the conditioned dentin [3].It has been shown that degradation of the adhesive joint begins by hydrolysis of denuded collagen fibrils by some endogenous proteolytic enzymes such as Matrix metalloproteinases (MMPs) and cysteine cathepsins [4,5].Caries, acid etching of dentin, and even mildly acidic components in adhesive resins (proanthocyanidin has a pH of 1.9) can transform the inactive form of MMPs to active proteases resulting in the degradation of adhesive joint. Various synthetic and natural agents have been shown to offer increase the bond stability of adhesive resins to dentin. As the world is on 'Go Green' crusade, dentistry too is thinking green. Research are being carried out testing various natural products to be used in the fields of conservative dentistry and endodontics. The aim of this study is to test the following null hypothesis:

1.Chlorhexidine (CHX) and grape seed extract (GSE) affects the shear bond strength (SBS) of resin modified glass ionomer cement (RMGIC) after 24 hours.

2.Guava leaf extract and Aloe vera extract does not affect the shear bond strength (SBS) of resin modified glass ionomer cement (RMGIC) after 24 hours.

Materials and methods

50 noncarious human premolars were used in this in vitro study, extracted for orthodontic or periodontal reasons, and stored in a 0.5% chloramine T solution until used for the study. The occlusal enamel of the teeth was removed (Figure 1) from all extracted teeth with water-cooled high-speed diamond disc. Dentin surfaces of the specimens were conditioned with 10% polyacrylic acid for 20 s, rinsed, and dried to maintain a moist surface. The specimens were randomly divided into control, chlorhexidine, grape seed extract, aloe vera extract and guava leaf extract groups. The teeth were mounted in self curing acrylic resin (Figure 2). Sample size was 50 (n=50).

1.Preparation of grape seed extract: Grape seed extract was available as 150mg capsule (Healthy Origins, Mega Naturals BP, California). A 5% solution was prepared by dissolving the contents of the capsule in 3mL of distilled water. (Figure 3)

2.Preparation of aloe vera extract: Leaves of aloe vera were collected and washed properly. These are then dissected longitudinally to extract the colourless fleshly tissue and homogenized in a grinder. (Figure 3)

3.Preparation of guava leaves extract: Leaves of guava were dried in fresh open air protecting from direct exposure to sunlight. A 50 g of powdered leaves were taken into a beaker containing 500 ml of sterile distilled water. Hot water extract was prepared by heating this in a

water bath till reduced to about 125 ml which is about one-fourth of the original volume. After the complete evaporation of the water content from extract, the resulting liquid was filtered using filter paper. (Figure 3)

After conditioning, no treatment was done in the control group. In the Chlorhexidine group the tooth specimens were treated with 2% Chlorhexidine for 1 min and gently air dried. In the proanthocyanidin group, 5% grape seed extract solution was applied on the specimens for 1 min followed by a gentle air steam. In the aloe vera extract group, the allotted specimens were treated with the prepared extract for 10 min and gently air dried. For group V i.e., guava leaf extract group, tooth specimens were treated with the extract for 10 min and gently air dried.

Then the RMGIC, was mixed according to manufactures instructions to restorative consistency and placed on the cut surface of the tooth and light cured for 30s. Then the specimens were stored in distilled water at 37˚C for 24 hours before shear bond strength test. (Figure 4 and 5)

Results

Means and standard deviations of shear bond strength in the control and study groups after 24 hours are reported in (Table 1). One way ANOVA test, (Table 2) revealed statistically significant difference between the groups regarding bond strength ($p < 0.05$).

Pairwise analysis using Tukey's HSD posthoc test, (Table 3) reported statistical significance between all pair groups ($p<0.05$) except control and grape seed extract groups ($p>0.05$). SBS values for chlorhexidine and guava leaf extract groups were lower compared to control. There was significant increase in the SBS values of aloe vera extract group compared to control.

Table 1- DESCRIPTIVE DATA OF BOND STRENGTH VALUES

Groups	N	Mean	Std. deviation	Std. error	95% Confidence Interval for Mean		Min.	Max.
					Lower Bound	Upper bound		
Group I (Control)	10	3.14	0.72	0.23	2.35	3.65	2.0792	4.121
Group II (Grape seed extract)	10	3.01	0.59	0.11	2.45	3.27	2.0713	4.0792
Group III (Chlorhexidine)	10	1.57	0.48	0.06	1.12	1.75	0.896	2.479
Group IV (Aloe vera extract)	10	4.92	0.73	0.57	4.11	5.42	3.9723	6.0797
Group V (Guava leaf extract)	10	0.81	0.35	0.04	0.57	0.98	0.3214	1.313

TABLE 2-SUMMARY OF ANOVA STATISTICS

	Sum of squares	DF	Mean square	F	P value
Between groups	100.66	4	25.166		
Within groups	15.77	45	0.35	71.809	0.0001*
Total	116.436	49			

*P<0.05 is statistically significant

TABLE 3- TUKEY'S HSD POSTHOC TEST (PAIRWISE COMPARISON)

Group (I)	Group (J)	Mean difference (I - J)	95% of confidence interval		P value
			Lower	upper	
G-I	G-II	-0.13	-0.8823	0.6223	0.987
	G-III	-1.57	-2.3223	-0.8177	0.0001*
	G-IV	1.78	1.0277	2.5321	0.0001*
	G-V	-2.33	-3.0823	-1.5777	0.0001*
G-II	G-III	-1.44	-2.1923	-0.6877	0.0001*
	G-IV	1.91	1.1577	2.6623	0.0001*
	G-V	-2.2	-2.9523	-1.4477	0.0001*
G-III	G-IV	3.35	2.5977	4.1023	0.0001*
	G-V	-0.76	-1.5123	-0.0077	0.046*
G-IV	G-V	-4.11	-4.8623	-3.3577	0.0001*

*P<0.05 is statistically significant

Discussion

In this study effect of various agents (natural and synthetic) on the shear bond strength of resin modified glass ionomer cement was investigated for a period of 24 hours.

The first was 5% grape seed extract, which is a natural collagen cross linking agent. Proanthocyanidin is the active content in grape seed extract which is responsible for its cross-linking property with the collagen fibers. Proanthocyanidin was available as a 150 mg capsule from Mega Natural-BP. It was extracted from Vitus vinifera seed which contains 90% polyphenols. Proanthocyanidin-collagen interactions may be explained via four mechanisms viz., covalent interactions, ionic interactions, hydrogen bonding or hydrophobic interactions [6-8]. Free phenyl hydroxyl groups of proanthocyanidin forms hydrogen bonds with the side chains of hydroxyl, carboxyl, amino groups of the collagen molecules thereby imparting stability to proanthocyanidin- collagen complexes [9]. Thus, the acid base reaction of RMGIC proceeds uninterrupted and the stiffening effect exerted by proanthocyanidin enables the penetration of resin component of RMGIC reinforcing micromechanical retention [10].

Presence of hybrid layer and formation of resin tags have been reported at the RMGIC tooth interface even though resin component in RMGIC is less and polyacrylic acid present exposes fewer collagen fibrils compared to phosphoric acid [11,12]. The hydrolysis of the hybrid layer may occur by endogenous MMPs [12]. MMPs are Zn2+ and Ca2+ dependent endogenous proteases which are present as inactive proenzymes. However, at the event of an acidic challenge these MMPs gets activated and brings about collagen degradation. Apart from functioning as a collagen cross-linking agent proanthocyanidin also functions as a non- specific MMP inhibitor thus making collagen fibrils resistant to biodegradation and resin-dentin bonds durable [13].

The values of shear bond strength obtained in this study were similar to that of the control group and statistically insignificant.

Shafiei et al., investigated the effect of carbodiimide and proanthocyanidin, on immediate and medium term bond strength of a resin modified glass ionomer and suggested proanthocyanidin as a matrix metalloproteinase inhibitor before bonding of resin modified glass ionomer to dentin [10].

Effect of 2% chlorhexidine on the shear bond strength was analyzed in this study. Chlorhexidine effectively prevents hydrolysis of the collagen fibrils by matrix metalloproteinase (MMP-2, -8, -9) and cysteine cathepsin and the subsequent hybrid layer degradation. However, no cross-linking action of chlorhexidine has been observed since there is no chemical interaction between chlorhexidine and collagen fibrils.

The shear bond strength was significantly lowered with application of chlorhexidine before bonding of resin modified glass ionomer to dentin in this study.

Dursun et al., investigated the effect of chlorhexidine on the shear bond strength of RMGIC and found that preconditioning with polyacrylic acid did not improve the bond strength of RMGIC after 24 hours, 6 and 12 months [14]. This was explained as follows. Chlorhexidine due to its cationic properties may react with carboxyl groups present in RMGIC which is anionic in nature. This delays the calcium-carboxyl interactions reducing dentin bonding capacity of the material. Maturation of the set matrix is also affected as chlorhexidine may also compete with aluminium ions for carboxyl groups of RMGIC [14]. Sanders et al., in his study also reported low physical properties of RMGIC when it is combined with chlorhexidine [15].

Another agent tested to investigate its effect on shear bond strength of RMGIC was aloe vera extract. Aloe barbadensis miller is claimed for its antibacterial, antifungal and antioxidant properties. In this study leaf extract of aloe vera was used as its parenchyma tissue

contains many substances such as polyphenols, indoles, alkaloids etc. [16] which may contribute towards its collagen cross linking effects. The shear bond strength was significantly improved with application of aloe vera extract before bonding of resin modified glass ionomer to dentin in this study.

Guava leaf extract was also tested for its effect on shear bond strength of RMGIC. This medicinal plant is a potent antioxidant as it contains several phenolic compounds such as gallic acid, ellagic acid, quercetin etc. [17]. However, this present study found lowered shear bond strength values with application of guava leaf extract before bonding of resin modified glass ionomer to dentin in this study.

Therefore, the first null hypothesis was proved wrong, and the second null hypothesis was partly accepted.

This preliminary study revealed the use of aloe vera leaf extract as a potential cross-linking agent and aiding in the maturation of RMGI bond. However, longer periods of aging are required to study its effects on Matrix metalloproteinase.

Conclusions

Within the limits of this study application of aloe vera extract before bonding of resin modified glass ionomer to dentin significantly improved the shear bond strength of RMGIC-dentin bonds.

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Legend Figures

Figure1: Occlusally reduced tooth specimens

Figure2: Mounted tooth specimen

Figure3: Surface treatment agents from right- Grape seed extract, Chlorhexidine, Aloe vera extract, Guava leaf extract

Figure 4: Shear bond strength testing in universal testing machine

Figure 5: Bond failure