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A comparative evaluation of micro-leakage in class v cavities restored with glass ionomer cement with or without incorporation of chitosan - An in vitro study

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Abstract

One of the challenging restorations for any clinicians are the class V cavities. Selecting a restorative material for such a lesion is challenging and technically demanding as the aetiology and progression of such lesion is multifactorial coupled with limitations in implementing preventing protocols, difficulties in isolation and bonding to root dentin. GIC (FUJI TYPE 2) is a material of choice for these lesions as it has modulus of elasticity similar to dentin, less technique sensitive, has re-mineralising effect and antiplaque properties. To enhance its anti-bacterial action, attempts have been made to incorporate agents like chlorhexidine to conventional GIC. There have been some preliminary studies that have shown improved mechanical properties of modified GIC when 10% v/v Chitosan was added to the liquid phase. Chitosan is a natural linear bio-polyaminosaccharide formed by alkaline deacetylation of chitin, which occurs naturally in the shells of crabs and shrimps and it is considered to be one of the most widely distributed biopolymers. In addition to its unique biological characteristics, such as biocompatibility, mucoadhesion, it has got a wide spectrum of antibacterial and antibiofilm properties against Gram-positive and Gram-negative bacteria. Micro-leakage studies has been used to evaluate the success of any restorative material used in the oral cavity since it is a measure of efficacy of marginal seal and a major contributing factor to secondary caries. The test results demonstrate the mean Micro Leakage values between GIC and 0.2% Chitosan groups. The mean Micro Leakage values for 0.2% Chitosan group [1.79 ± 0.69] was significantly lesser as compared to GIC group [2.72 ± 0.86] with a mean difference of 0.93 [95% CI, 0.62 – 1.23].

Keywords: Type II GIC, Chitosan, Class V Cavity, Microleakage

Introduction

Success of any restoration depends on the marginal integrity of the restorative material to the tooth structure. One of the challenging restorations for any clinicians are the class V cavities. Selecting a restorative material for such a lesion is challenging and technically demanding as the aetiology and progression of such lesion is multi-factorial coupled with limitations in implementing preventing protocols, difficulties in isolation and bonding to root dentin. 1 Therefore in such situations, one prefers a material that is less technique sensitive, have low modulus of elasticity to allow the restoration to flex with the tooth and also have less propensity for plaque accumulation.[2]

The stresses that are generated at the tooth-restoration interface often exceeds the bond strength of the restorative material leading to microscopic gaps that eventually cause micro-leakage and consequent marginal discoloration, postoperative sensitivity, secondary caries leading to pulpal damage.3 Many studies have shown that there is greater amount of bacteria and plaque accumulating on the surface of cervical lesions which limit the longevity of these restorations.4 Hence a restorative material with antibacterial property would inactivate bacteria and prevent recurrent caries.

GIC (FUJI TYPE 2) is a material of choice for these lesions as it has modulus of elasticity similar to dentin, less technique sensitive, has remineralising effect and antiplaque properties.5 In addition, the release of fluoride may provide some protection of the tooth surface against developing caries. However, the major concern about this material is, compromised mechanical properties and dissolution in the oral cavity; even the antibacterial effect of conventional GICs was not considered effective enough to reduce bio film formation and cell viability.[6]

To enhance its anti-bacterial action, attempts have been made to incorporate agents like chlorhexidine to conventional GIC. While it improved the antibacterial property, it adversely affected its mechanical and physical properties.5 Thus, there was a need for an alternative biocompatible additive which has a potential for enhancing the antibacterial effect of GIC without compromising its physical properties.

There have been some preliminary studies that have shown improved mechanical properties of modified GIC when 10% v/v Chitosan was added to the liquid phase.8 Chitosan is a natural linear bio-polyaminosaccharide formed by alkaline deacetylation of chitin, which occurs naturally in the shells of crabs and shrimps and it is considered to be one of the most widely distributed biopolymers. In addition to its unique biological characteristics, such as biocompatibility, mucoadhesion, it has got a wide spectrum of antibacterial and antibiofilm properties against Gram- positive and Gram-negative bacteria. [7] Marginal integrity is one of the important property required for success of any restoration.

Micro-leakage studies has been used to evaluate the success of any restorative material used in the oral cavity since it is a measure of efficacy of marginal seal and a major contributing factor to secondary caries.[9] As there are no studies found in the literature on the marginal integrity of chitosan incorporated GIC, this study was undertaken to investigate the effect of chitosan modified glass ionomer restorative material on micro-leakage in class V cavities when compared to unmodified Glass ionomer cements.

Objectives

1. To assess micro-leakage of unmodified Glass Ionomer restorations (Fuji type II) in class V cavities.
2. To assess micro-leakage of 0.2% Chitosan incorporated Glass Ionomer restorations in class V cavities.
3. To compare the micro-leakage of unmodified Glass Ionomer restorations with 0.2%chitosan modified Glass ionomer restorations in class V cavities.

Materials and methods

Sample size was calculated using G-power software version 3.0.10 by placing the following inputs in the software.

Test: Mann – Whitney test Input

Effect size d = 0.5

α err prob = 0.05 power (1 – β err prob) = 0.80

Allocation ratio N2 /N1 = 1 Output

Sample size group 1 Sample size group 2 Total sample size = 53 = 53 =106

A total of 53 freshly extracted human maxillary first premolar teeth were collected from the department of oral and maxilla-facial surgery, Dayananda Sagar College of dental sciences Bengaluru, after taking consent from the patient.

Selection Criteria

- Inclusion criteria: Freshly extracted intact human permanent maxillary premolar teeth.
- Exclusion criteria: Teeth with evidence of dental caries Teeth with any fracture or craze lines Teeth with developmental anomalies Teeth with previous restoration Teeth with endodontic treatment

Materials

- 53 extracted human maxillary premolar teeth Airotor handpiece and burs
- Micromotor
- Glass ionomer cement- Fuji Type II
- 0.2% Chitosan
- Methylene blue dye

Methodology

53 freshly extracted permanent human maxillary premolar teeth were used for this study. Each tooth was numbered and two cavities prepared on the buccal and lingual surfaces respectively with standardized dimensions being 4.0 mm in width, 3.0 mm in height, and 2 mm in depth using a marked 245 bur with airotor handpiece giving a total of 106 samples.

The dimensions of the prepared cavity were checked using a periodontal probe.

Self-Curing GLASS IONOMER CEMENT- Fuji Type II and the experimental cement was formulated by incorporating 0.2% chitosan in the liquid component of GIC.

The samples were numbered and randomly allocated to the respective groups using lottery method into two groups. Teeth were restored according to the allocated groups, and sectioned mesio-distally to get specimen of both groups from the same substrate. (n= 106)

Group 1: Cavity with GIC (Fuji type II) n=53

Group 2: Cavity with chitosan incorporated GIC (Fuji type II) n=53

After placement of the restorations, all teeth were stored in de-ionized water at 37 °C for 24 hours.

The teeth were then subjected to 500 thermal cycles between 5±2o C−55±2o C water baths, with a dwell time of 30 seconds and transfer time of 3 seconds [Malekipour et al., 2010; Mortazaviet al., 2011; Khier and Hassan, 2011].

After thermo-cycling, all teeth were stored in distilled water at 37° C for 24h to prevent dehydration.

The teeth were covered with two layers of nail varnish, except GIC and chitosan modified GIC restoration 1mm area around it, followed by immersion in 0.6 % aqueous methylene blue dye for 48 hours. The specimens were rinsed and sectioned mesio-distally to separate the two groups.

For micro-leakage study each samples were sectioned Bucco-lingually and Micro leakage test was done using dye penetration technique by calibrated examiners using SEM at CMTI, Bengaluru.

The micro-leakage scores were obtained in the micrometres from the confocal microscopic analysis for each sample and analysed to get an accurate and reliable results.

The extent of the micro-leakage was noted according to the following scoring criteria[10]

1. No marginal leakage
2. Up to 1/3 cavity depth
3. 1/3-2/3 cavity depth
4. >2/3 cavity depth but not involving the axial wall.
5. Involving the axial wall.

Statistical analysis: Statistical Package for Social Sciences [SPSS] for Windows, Version 22.0. Released 2013. Armonk, NY: IBM Corp., was used to perform statistical analyses.

Descriptive Statistics: It includes expression of the micro leakage values for different study groups in terms of mean & SD.

Inferential Statistics: Mann Whitney U Test was used to compare the mean micro leakage values between GIC and 0.2% Chitosan groups.

The level of significance [P-Value] was set at P <0.05.

Results

Comparison of mean Micro leakage values between GIC and 0.2% Chitosan using Mann Whitney U Test

Groups	N	Mean	SD	Mean Diff	95% Conf. Interval		P-Value
					Lower	Upper	
GIC	53	2.72	0.86	0.93	0.62	1.23	<0.001*
0.2% Chitosan	53	1.79	0.69				

Table 1

Fig 1: class v cavities prepared on teeth.

Fig 2: chitosan and GIC type ii

Figure 3:

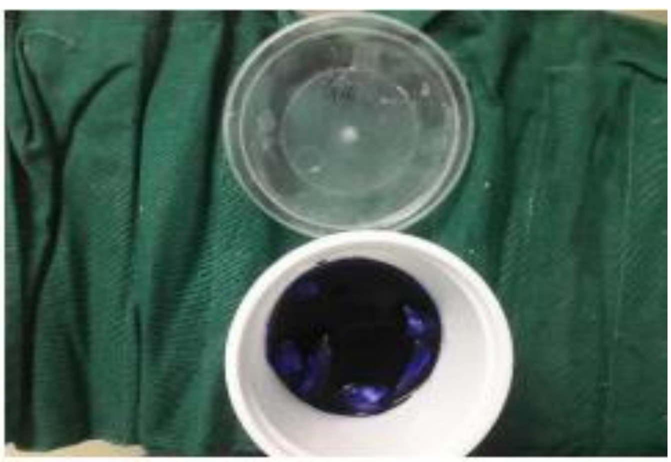

Fig 4: sampled dipped in methylene blue

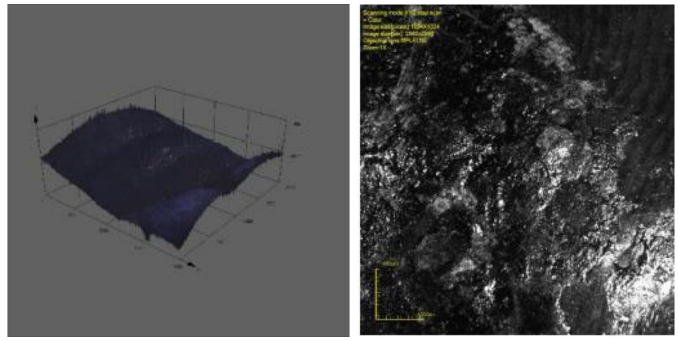

Fig 5: GIC under the laser confocal microscope.

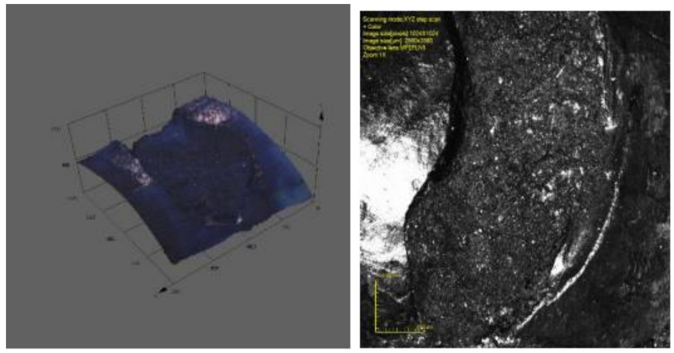

Fig 6: 0.2% chitosan modified GIC under the laser confocal microscope.

The test results demonstrate the mean Micro Leakage values between GIC and 0.2% Chitosan groups. The mean Micro Leakage values for 0.2% Chitosan group [1.79 ± 0.69] was significantly lesser as compared to GIC group [2.72 ± 0.86] with a mean difference of 0.93 [95% CI, 0.62 – 1.23]. This mean difference in the mean micro leakage values between GIC and 0.2% Chitosan group was statistically significant at $P<0.001$.[Refer figure no.1]

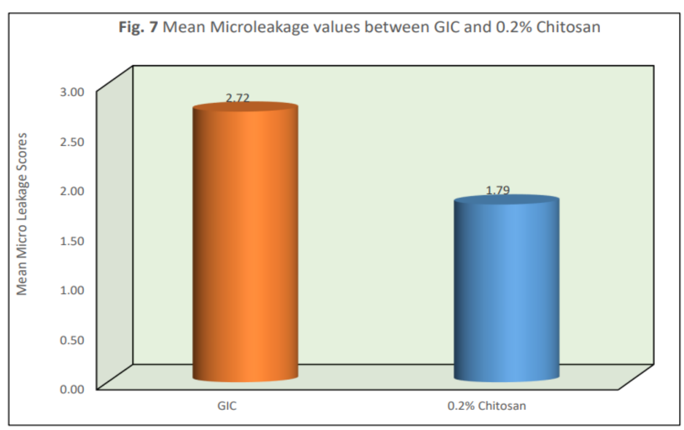

Fig 7:

Discussion

The success of any restoration depends on the size and positioning of the cavity margins. The depth of the lesion is an important factor in determining not only the pulpal status but also to bring about certain degree of standardization in the type of dentin substrate. Literature reviews summarized that most cervical lesions are moderately deep and may have symptoms of sensitivity to sweets, cold or air. In our study to mimic the moderately deep lesion, cavity dimension was standardised based on the study by Deena et all.

GIC and micro hybrid composites are the materials of choice due to their low modulus of elasticity which allow the restoration to flex with the tooth. However greater number of bacteria and plaque accumulation are noticed on the surface of composites and enamel demineralization occurs owing to plaque accumulation, compared to other restorative materials, leading to secondary caries. GIC with its fluoride releasing property is considered a better choice of materials in such cases.

Although it has anti-bacterial properties its mechanical and physical properties still has scope for improvement. Many materials have been incorporated into GIC, out of which chitosan is an attractive agent as it's a natural biopolymer and has good antimicrobial property too. Its incorporation in the restorative materials has shown improved physical and mechanical properties. Any substance added, should not alter or deteriorate the property of the parent material, hence this study was done to evaluate the marginal adaptation of the combined restorative material through micro-leakage study.

The results of this study showed that the incorporation of 0.2% chitosan did affect the micro-leakage of GIC wherein it resulted in lesser micro-leakage compared to the unmodified GIC by a significant difference. GICs set by an acid base reaction. The setting reaction initiates with the mixing of glass powder and poly-alkanoic acid. During the mixing process, the surface of the glass attacked by the poly-alkanoic acid and degrades, followed by Ca+2 and Al+3 ions are leached into aqueous medium.

A firm gel is formed when these ions form complexes with the acid. In the hardening stage, the silica and phosphate ions released from the glass condensate and form a gel matrix. The presence of phosphorus and silicon in the matrix of glass ionomers suggest that these elements form an inorganic network within the matrix of GICs. Two kinds of networks have been observed in set cement structure, the organic network that consists of polyacid chains (copolymers) with metallic connecting ions (polysalt bridges) and the inorganic net- work based on silica and phosphate ions.

Debnath et al.[11] investigated the effect of modifying the liquid phase of conventional GICs with 10% CH (by volume) on the antimicrobial properties and adhesion to enamel in comparison to conventional GICs. The results showed that the modification of the liquid phase of conventional GICs with CH significantly enhanced the antimicrobial properties of conventional GICs against S. mutans and increased the bond strength of GICs to enamel. These results are in accordance with the study by Mishra et al.[12] who showed an increase in the antimicrobial activity and marked increase in the compressive and flexural strength.

The possible explanation for this finding could be related to the bonding between the cement and enamel which is achieved through the polar and ionic attraction between the carboxylic groups of polyacrylic acid and hard tooth surfaces. Since CH is a high chelating agent, more polyacrylic acid chains would diffuse into the enamel to displace the phosphate and calcium ions from hydroxyapatite crystals, thus lead to the development of an ion enriched layer of cement that is firmly attached to the tooth. This could also explain the reduced micro-leakage seen with modified GIC in our study.

Denise F.S. Petri et al[13] early in 2007, showed that the flexural strength of a commercial GIR can be considerably improved by the addition of a tiny amount of CH where the addition of 0.2% chitosan in the liquid led to the highest flexural strength among the concentrations used. Furthermore, the enhanced antimicrobial effect could be explained by the following mechanisms

1. CH promote displacement of Ca2+ of the anionic sites of the membrane of bacteria, thus resulting in cell damages.

2. Interaction between positive load of CH and negative load of microbial cell wall, which might lead to rupture and loss of important intracellular constituent. Chitosan with low molecular weight and viscosity could possibly penetrate inside the bacterial cell thus inhibiting the transcription and translation of bacterial DNA.14

It appears that the chitosan modified GIC is an exciting combination, beneficial especially in Class V Cavities. In Our study even lower concentration of 0.2% chitosan added to GIC showed reduced micro-leakage. Hence, it could be seen as a promising modification of already existing GIC to emerge as a new restorative material for permanent restoration of Class V lesions.

However, further in-vitro and long-term in vivo studies with regards to its antibacterial effectiveness, water sorption, solubilty, and long-term stability together with refinement in formulation technique if necessary before it can be recommended for clinical usage.

Clinical Implications

Based on the results of our study and that found in the literature, it is evident that chitosan incorporated GIC in addition to being antibacterial, seem to have improved mechanical properties and stable bonds as compared to unmodified GIC. Considering the above advantageous property of this material, their use may be clinically useful in restoring class V cavities with high caries risk.

Conclusion

Within the limitations of this present in-vitro study, 0.2% chitosan modified GIC showed statistically significant reduction in the micro-leakage when compared to the conventional unmodified GIC. Chitosan has been a new and upcoming material in the field of dentistry offering a plethora of advantageous properties when added to other materials without altering the physico-chemical properties of the material in a deleterious manner.

Our study was able to conclude that the addition of small concentration (0.2%) of chitosan to Type II GIC enhanced the micro-leakage property of the material and this can be beneficial while restoring Class V cavities with the added the advantage of improved flexural strength and anti-microbial properties as well. However, further in-vitro and long term in-vivo studies with regards to its antibacterial effectiveness, water sorption, solubility, and long-term stability together with refinement in formulation technique if necessary before it can be recommended for clinical usage.

Objectives achieved

- Micro-leakage values determined for both GIC and chitosan modified GIC.
- Comparative evaluation of unmodified GIC and modified GIC.

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