

# *In-vitro* Comparative Evaluation of Cariostatic Potential and Marginal Microleakage of Commonly-used Glass-Ionomer Restorative Materials as Interim Therapeutic Restorations

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## Abstract

*In-vitro* Comparative Evaluation of Cariostatic Potential And Marginal Microleakage Of Commonly-Used Glass-Ionomer Restorative Materials as Interim Therapeutic Restorations.

**Introduction:** Glass ionomer cements (GIC) can ideally serve many restorative purposes in paediatric dentistry as it has advantages of chemical adhesion to tooth structure, as well as fluoride release and uptake into hard tissues.

**Aims:** To compare four commercially available conventional GIC based on the amount of fluoride release, marginal integrity and ability to increase microhardness of underlying artificial dentinal caries via remineralization.

**Methods and Material:** Standardized cavities were prepared on the buccal surface of 60 extracted sound premolars and artificial caries were induced onto the dentinal floor. Specimens randomized into four groups of fifteen teeth and restored with Fuji VII<sup>®</sup>, Riva Protect<sup>®</sup>, Riva Self Cure<sup>®</sup> and Fuji IX GP<sup>®</sup> Extra respectively. Measurement of fluoride release was done for 60 days. Subsequently, similar restorations prepared on lingual surface for microleakage test according to standard protocols. Dentin underlying buccal restorations were subjected to microhardness test while lingual restorations were evaluated for marginal microleakage.

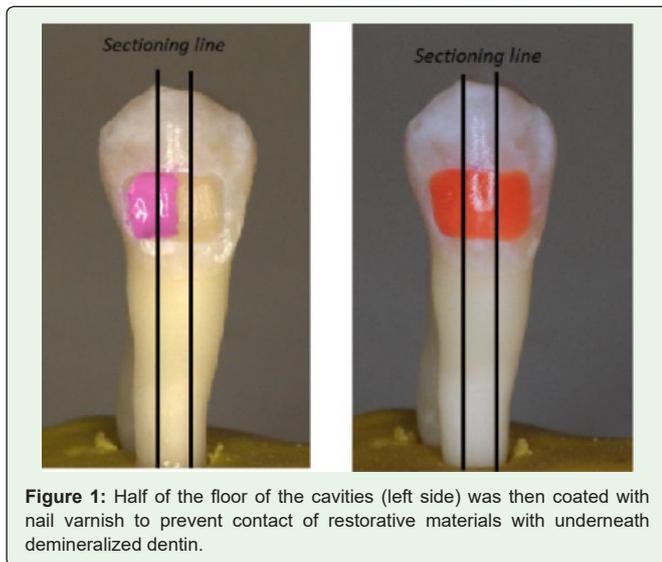
**Results:** One-way ANOVA statistical analysis revealed significant difference was found in the amount of fluoride release between materials on all days of measurement ( $p < 0.05$ ). Riva Protect<sup>®</sup> released the highest amount of fluoride, followed by Fuji VII<sup>®</sup>, Riva Self Cure<sup>®</sup> and Fuji IX GP<sup>®</sup> Extra. Wilcoxon signed-rank test showed Riva Protect has the ability to significantly improve the microhardness of artificial caries to the depth of 100  $\mu\text{m}$  ( $p < 0.05$ ). There was a significant difference in the marginal sealing ability between materials with more microleakage seen with low-viscosity materials, analysed by Pearson's Chi-Square test.

**Conclusions:** All materials possess cariostatic potential. However, Riva Protect<sup>®</sup> is suggested to exert the greatest cariostatic effects among the test materials.

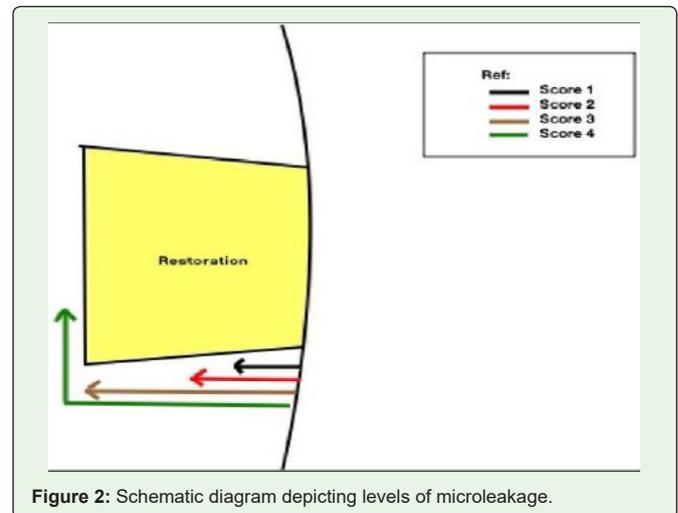
## Introduction

Glass Ionomer Cements (GIC) have been widely used in paediatric dentistry mainly as interim therapeutic restorations (ITR). In accordance to the concept of Minimally Invasive Dentistry (MID), placement of an ITR can be aimed at arresting caries progression by remineralization, thus eliminating the need for a more invasive definitive treatment later on. Remineralization of residual dentinal caries can be achieved by sealing off the cavity leading to depletion of the cariogenic potential of the remaining bacteria, and remineralizing the structure by diffusion of ions from material placed on the restored cavity [1,2]. Uptake of the leached fluoride ions into adjacent dental hard tissues resulting in remineralization dentinal lesions have been demonstrated both *in vitro* and *in vivo* [1,3-4]. However, not many studies have investigated the improvement of quality of affected dentinal lesions after restorations with glass ionomers. By measuring microhardness of dentin, it is possible to indirectly evaluate its mineral content qualitatively, and assess the improvement of its mechanical resilience as a result of ions incorporation from a restorative material. Maneenu et al. suggested that the degree of remineralization of demineralized dentin may depend on properties of the restorative materials, where they observed more hardness improvement in dentin restored with higher-fluoride releasing material [3].

A review of the literature confirmed that it is acceptable to leave soft and wet dentin at the base of cavity preparations, provided a complete seal is established and maintained [5]. Generally, GIC restorations have good marginal adaptation to tooth structure. However, there is lack of information available in the literature on marginal sealing ability of newer materials such as Riva Protect and Riva Self Cure for comparisons among other conventional counterparts. Commercially available GIC products vary considerably in their ability to release fluoride [6-8]. As cariostatic



**Figure 1:** Half of the floor of the cavities (left side) was then coated with nail varnish to prevent contact of restorative materials with underneath demineralized dentin.



**Figure 2:** Schematic diagram depicting levels of microleakage.

effects of a material are thought to be related to the amount of their fluoride release, GICs have undergone continuous development, improvement, and diversification in order to meet the restorative and therapeutic demands. Therefore, there is a need to evaluate these materials to determine the extent of their cariostatic potential. This is important to help the clinicians to make a choice regarding which material that may be best served as interim therapeutic restorations.

The aims of this study were to comparatively evaluate the cariostatic potential of recently available conventional GICs against a commonly-used glass ionomer cements stabilizing material, GC Fuji VII<sup>®</sup> based on their amount of fluoride release, marginal integrity and ability to remineralize artificial dentinal caries.

**Table 1:** List of materials used as restorations and their composition.

Groups	Materials	Manufacturer	Composition (Wt.%)
Group 1 (n = 15)	Fuji VII <sup>®</sup> Self-cure, encapsulated	GC Asia, Japan	Compartment 1 (powder) Fluoro Aluminosilicate Glass -100 Iron (III) oxide – <0.1 Compartment 2 (liquid) Polyacrylic acid - 40 Distilled water - 50
Group II (n = 15)	Riva Protect Self-cure, encapsulated	SDI Limited, Australia	Compartment 1 (powder) Fluoro Aluminosilicate Glass -90 Polyacrylic Acid – 10 Compartment 2 (liquid) Polyacrylic acid - 25 Tartaric Acid - 10 Balanced ingredient – 65
Group III (n = 15)	Riva Self Cure Self-cure, encapsulated	SDI Limited, Australia	Compartment 1 (powder): Fluoro Aluminosilicate Glass - 90-95 Polyacrylic Acid - 5-10 Compartment 2 (liquid): Polyacrylic - 20-30 Tartaric acid – 10-15
Group IV (n = 15)	Fuji IX GP <sup>®</sup> Extra Self-cure, encapsulated	GC Asia, Japan	Compartment 1 (powder): Fluoro Aluminosilicate Glass – 90-95 Polyacrylic Acid – 5-10 Compartment 2 (liquid): Polyacrylic acid – 30-40 Proprietary ingredient – 5

### Materials and Methods

This is an *in-vitro* experimental study carried out from June 2014 until January 2015 at Faculty of Dentistry, National University of Malaysia. Sixty sound human permanent premolar teeth, extracted for orthodontic purposes were utilized. After extraction, the teeth were cleaned, polished and disinfected with 70% alcohol solution. A sample size of 15 teeth per group was calculated to provide 80% power with  $\alpha=0.05$  two-sided significance level. For restorative marginal microleakage test, each tooth was further sectioned buccolingually to produce a total of 8 restorative margins for each tooth. Thus, each group consisted of 120 samples of restorative margins for evaluation.

### Induction of artificial dentinal caries

All teeth were drilled on buccal surfaces to create a standardized Class V cavity size of 2 mm depth, 4 mm length and 2 mm width using high-speed diamond flat end cylinder bur. Each cavity was measured with William’s graduated probe to ensure uniform size. The entire surface of all teeth were coated with acid-resistant nail varnish, to leave behind only the floor of the cavities onto where an artificial dentine caries lesion was induced by immersion in 30 ml of 50 mM acetate buffer demineralizing solution, containing 2.2 mM calcium chloride (CaCl<sub>2</sub>), 2.2 mM potassium phosphate (KH<sub>2</sub>PO<sub>4</sub>), at pH 5.0 for seven days [9-10] and incubated at 37°C. The artificial dentine caries induced by this protocol will produce a dentine demineralization of around 130- 190  $\mu$ m lesion depth that simulate caries-affected dentine [11]. After the demineralization period, the nail varnish was scraped off in preparation to receive restorations. Half of the floor of the cavities (left side) was then coated with nail varnish to prevent contact of restorative materials with underneath demineralized dentin (Figure 1). This area of varnish-coated dentin was served as a control for baseline microhardness.

### Grouping and randomization

The four materials used in this study were GC Fuji VII<sup>®</sup>, SDI Riva Protect<sup>®</sup>, SDI Riva Self Cure<sup>®</sup> and Fuji IX GP Extra<sup>®</sup> (Table 1). All teeth with artificial dentinal caries were randomly divided into four groups (15 teeth per group) with Group 1 acts as a control. After restorations, specimens were placed inside individual vials containing 10 ml of deionized water.

**Table 2:** Multiple comparisons revealed a significant difference between fluoride release between the different viscosity of materials.

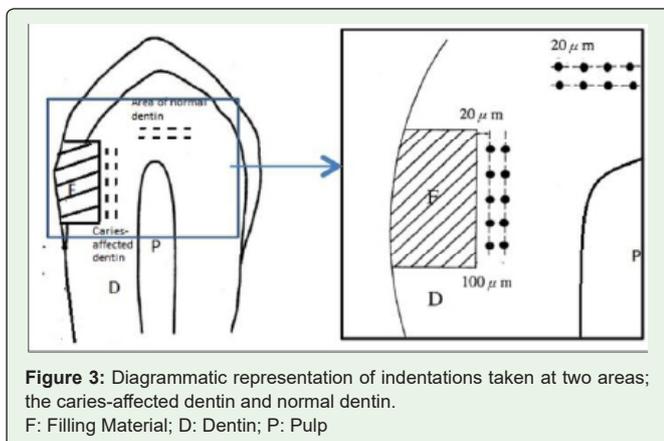
Day of measurement	Comparison groups	Mean difference	p-value
Day 1	Fuji VII® and Fuji IX GP® Extra	0.1700	0.016*
	Riva Protect and Riva SC	0.4300	<0.001**
	Riva Protect and Fuji IX GP® Extra	0.6000	<0.001**
Day 3	Fuji VII® and Fuji IX GP® Extra	0.4300	<0.001**
	Riva Protect and Riva SC	0.2800	<0.001**
	Riva Protect and Fuji IX GP® Extra	0.3700	<0.001**
Day 7	Riva Protect and Riva SC	0.2447	<0.001**
	Riva Protect and Fuji IX GP® Extra	0.3567	<0.001**
Day 13	Fuji VII® and Fuji IX GP® Extra	0.2300	0.036*
	Riva Protect and Riva SC	0.1840	<0.001**
	Riva Protect and Fuji IX GP® Extra	0.1607	<0.001**
Day 30	Fuji VII® and Fuji IX GP® Extra	0.1700	0.032*
Day 60	Riva Protect and Riva SC	0.1600	0.046*

**Fluoride release measurement**

The fluoride release from all groups was measured on day 1 (24 hours after restorations), day 3, day 7, day 14, day 30 and finally at day 60. The solution was renewed after each measurement. Fluoride ion concentration in the samples was measured using spectrometer (DR 3900 HACH) which was calibrated before each measurement using five standard fluoride solutions ranging from 0.05 to 5.00 ppm fluoride.

**Preparation for microleakage test**

After 60 days of fluoride measurement, all teeth were prepared with Class V cavity on the lingual surfaces with the standardized size of 3mm width x 2mm length x 2mm depth. Each cavity on the lingual surface was restored with the same material as on the buccal surface, with similar cavity preparation. All restored teeth were stored in normal saline at 37°C for 2 days before subjecting them to thermocycling. This was done for 1500 cycles between baths of 5°C, 37°C, and 55°C, with a dwell time of 30 seconds. Then, the external surface of all samples was coated with varnish to seal the entire part of samples, except 1 mm of the periphery of the restorations. Specimens were immersed in 5% methylene blue for 4 hours. Sectioning of specimens done in a buccolingual direction to divide both buccal and lingual restorations surface area into three parts using a diamond saw, Isomet 4000 (Buhler, Illinois, USA). This gave four sections from each tooth that revealed both buccal and lingual restorations which were evaluated for microhardness test and marginal microleakage test respectively.



**Figure 3:** Diagrammatic representation of indentations taken at two areas, the caries-affected dentin and normal dentin. F: Filling Material; D: Dentin; P: Pulp

**Table 3:** Total cumulative fluoride release from materials.

Types of Materials	Total Cumulative Fluoride Release (mg/l)	p-value
Fuji VII®	7.24	<0.001*
Riva Protect	7.46	
Riva SC	5.93	
Fuji IX GP® Extra	5.75	

**Evaluation for microleakage at restorative margin using stereomicroscope**

Four cross-sections were obtained through the lingual restorations, exposing eight restorative margins for microleakage evaluation (eight samples per specimen). The degree of dye penetration in the restorative-cavity walls was assessed under a stereomicroscope at 40x magnification and measured using the scoring criteria described by Khera and Chan [4,12] as in Figure 2.

**Determination of dentin surface microhardness**

Surface microhardness was analysed on dentin underlying buccal restoration of lateral sections. Five indentations at 20 µm intervals were made in each of two columns at depths of 20 and 100 µm from the bottom of the cavity towards pulp (Figure 3). Dentin surface microhardness was measured using a microhardness tester (HMV-2T Vickers Hardness Tester; Shimadzu Corporation, Tokyo, Japan) with a load of 10g applied for 10 seconds for demineralized dentin and 25g for 10 seconds for sound dentin. Ten average values of Vickers hardness were obtained for each section, and for each tooth, both values for control and test were obtained for comparisons.

**Data collection and statistical analyses**

Intra-Class Correlation Coefficient (ICC) analyses and Cohen’s Kappa test revealed a high level of intra-rater agreement on the measurement of indentation size and degree of microleakage respectively. Data analysed using the IBM SPSS Data Editor Version 22.0. The mean values for fluoride release on the pre-determined days of various groups were compared using One-way ANOVA. Wilcoxon signed-rank test was used to compare means of microhardness improvement after restoration with test groups. Pearson’s Chi-

**Table 4:** Comparisons of dentin microhardness (VHN) after restorations of various materials at distance of 20µm and 100µm from tooth-restorative interface.

Types of materials		Distance of 20 µm		Distance of 100 µm		Normal dentin (IQR)
		Median (IQR)	p-value	Median (IQR)	p-value	
Fuji VII®	Baseline	52.7 (45.5-57.8)	0.068	57.3 (52.9-61.3)	0.050	58.3 (56.9-59.7)
	Restored	53.1 (45.0-57.9)		57.8 (53.0-62.7)		
Riva Protect	Baseline	53.6 (48.2-60.6)	0.004*	60.0 (56.7-63.9)	0.007*	61.9 (57.8-63.2)
	Restored	56.7 (48.2-60.9)		61.7 (56.7-64.7)		
Riva Self Cure	Baseline	52.5 (46.9-55.0)	0.094	55.7 (52.3-59.0)	0.141	57.9 (55.7-59.2)
	Restored	52.9 (47.0-55.3)		55.8 (52.5-59.2)		
Fuji IX GP® Extra	Baseline	54.8 (48.4-59.5)	0.066	59.5 (55.2-62.3)	0.093	61.5 (58.9-62.2)
	Restored	55.5 (49.8-60.2)		59.9 (55.0-63.4)		

**Table 5:** Between groups comparison of number of restorations with degree of marginal microleakage.

Types of Materials	No. of Samples	Degree of microleakage, N (%)					p-value
		0°	1°	2°	3°	4°	
Fuji VII®	114	8 (7.0)	22 (19.3)	46 (40.4)	27 (23.7)	11 (9.6)	0.021*
Riva Protect	112	15 (13.4)	19 (17.0)	44 (39.3)	21 (18.8)	13 (11.5)	
Riva SC	116	24 (20.7)	30 (25.9)	41 (35.3)	14 (12.1)	7 (6.0)	
Fuji IX GP® Extra	110	25 (22.7)	26 (23.7)	38 (34.6)	16 (14.5)	5 (4.5)	
Total	452	72 (15.9)	97 (21.5)	169 (37.4)	78 (17.3)	36 (8.0)	

square test was used to evaluate the association between degree of microleakage and type of materials. The level of significance was set at  $p < 0.05$ .

## Results

### Measurement of fluoride release

A significant difference in the amount of fluoride release in between groups on all day of measurement ( $p < 0.05$ ) was observed. Multiple comparisons revealed a significant difference between fluoride release between the different viscosity of materials (Table 2). Total cumulative fluoride release for 60 days of measurement between materials was statistically significant, with greatest fluoride elution from Riva Protect® (Table 3). The pattern of fluoride release was typical for all materials as shown by the plot graph in Figure 4. All materials released the greatest amount of fluoride on day 1, followed by a rapid decline by day 3, and further decreased for subsequent days.

### Measurement of dentin surfaces microhardness

Statistically significant increase in dentin microhardness was observed after restorations of Riva Protect® at 20µm and 100µm depth from the tooth-restorative interface ( $p < 0.05$ ) as in Table 4.

### Comparison of microleakage at restorative margins

There was a statistically significant association between degree of microleakage at restorative margins with the type of the materials ( $p = 0.021$ ). Table 5 shows that samples from Fuji VII® and Riva Protect® had more microleakage at 2° and 3°, whereas Riva Self Cure® and Fuji IX GP Extra® samples had more microleakage at 1° and 2°. The least number of samples with 4° microleakage were from Riva Self Cure® and Fuji IX GP Extra®.

## Discussion

It has been well documented that fluoride release from GICs follows a typical pattern of initial “burst” followed by a rapid decline before reaching a low constant level [8] as similarly observed in this study. However, the amount of fluoride release from GICs may vary considerably as it is influenced by several intrinsic variables, such as formulation and fillers. In agreement with other studies [6-7], high viscosity GICs such as Riva Self Cure® and Fuji IX GP Extra® were found to release slightly less fluoride. A higher proportion of powder-to-liquid in high viscosity GICs provides more glass filler content

with fewer monovalent ions cross-linking the polymer chains holding them close together, leading to less water transport and, consequently less fluoride release. Furthermore, the presence of more pores, cracks and voids in the structure of the set cement of low-viscosity GICs such as Riva Protect® and Fuji VII® might allow more diffusion of fluoride ions, during the stable phase of release. In addition, Riva Protect® which utilizes the proprietary ion glass filler system with the addition of amorphous calcium phosphate was proven to release the greatest amount of fluoride compared to other tested materials. Nevertheless, the exact mechanism of this is unknown. These differences might be clinically relevant, especially in patients with high caries-risks and where residual caries may be present following cavity preparation.

Although both Fuji VII® and Riva Protect® similarly releases high fluoride to the external environment, significant improvement of dentin microhardness was observed only underneath the Riva Protect® up to the depth of 100µm. This might be explained by the enhanced formulation with the inclusion of calcium-amorphous nanofillers to Riva Protect®. It has been suggested that for net remineralization to occur, an adequate level of calcium and phosphate ions must be available [11,13]. Thus, the increased availability of calcium and phosphate ions released during the setting and maturation of Riva Protect® is suggested to enhance this remineralization process [10]. In line with this, Prabhakar et al. [10] also found that the incorporation of bioactive glass containing oxides of calcium, phosphorus and silicate into conventional glass ionomers and resin-modified glass ionomers was able to significantly enhance remineralization [13]. They suggested the potential of this bioactive glass as a filler component of restorative materials.

Another important factor to halt caries progression is the establishment of a good seal between residual caries in a tooth cavity and the oral environment. Therefore, an ideal restorative material should possess minimum leakage potential. Marginal microleakage of conventional GICs used in this study was relatively high. About 25.3% of total sample have microleakage of more than half of cavity depth. Other authors also reported that microleakage was evident but with a greater extent with conventional GICs, compared to resin-modified GICs and composites [12-14]. In the present study, low-viscosity GICs exhibited more microleakage compared to the high-viscosity variants. In contrast, Singla et al. found significantly more microleakage with Fuji IX GP® as compared to Fuji VII® which has been suggested to be due to its high viscosity, which did not allow proper wetting of the tooth surface thus preventing the formation of a good seal between tooth-restorative interface [14]. One possible explanation is that the texture of Fuji VII® and Riva Protect® were found on stereomicroscope photographs to be more granulated with many cracks and air voids, which might result in the leakage in the present study.

## Conclusion

All materials tested are suitable to be used as ITR as they release a significant amount of fluoride and able to remineralize the “affected” carious dentin, by means of improving its microhardness. Low-viscosity GICs were found to release more fluoride, whereas material with inclusion of amorphous calcium-phosphate such as Riva Protect® provides better remineralizing effects on the carious dentin. Despite the poor marginal sealing ability, the conventional GICs can still serve in the mouth as a temporary restoration due to its advantages of therapeutic properties.

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