ORIGINAL RESEARCH

Comparative Evaluation of Microleakage of Fiber-reinforced Glass Ionomer Cement and Conventional Glass Ionomer Cement Restorations Immersed in Three Different Beverages: *In Vitro* Study

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Abstract

Aim: The aim of the study was to compare and evaluate the microleakage of fiber-reinforced glass ionomer cement (GIC) and conventional glass ionomer cement restorations immersed in three different beverages.

Materials and methods: A total of 96 human exfoliating deciduous molars were selected which were cleaned and disinfected. Standardized Class V cavity preparations were done and buccal surface were restored with experimental fiber-reinforced glass ionomer cement (Exp-FRGIC), lingual surface were restored with Type II conventional GIC. They were divided into four groups according to the test beverage. The samples were subjected to various immersion regimes and evaluated for microleakage under stereomicroscope.

Results: Intragroup comparison for (Exp-FRGIC) showed significant microleakage when immersed in fresh fruit juice at high immersion whereas intragroup comparison in conventional GIC, showed highest microleakage with soft drink. Intergroup comparison of (Exp-FRGIC) in high immersion regime, showed more microleakage with specimen immersed in soft drink followed by fresh fruit juice.

Conclusion: It can thus be concluded that the three beverages used in the study affected the microleakage of both restorative materials significantly.

Clinical significance: Increasing usage of fruit juices in the pediatric diet has a definite impact on the progression of caries and it directly or indirectly affects the quality of restorations placed in the oral cavity.

Keywords: Beverages, Fiber-reinforced glass ionomer cement, Glass ionomer cement, Microleakage, Soft drinks.

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Introduction

There is no substitute for natural teeth, if lost once it's lost forever. No dental material introduced so far can be termed ideal. They can mimic the natural teeth in several ways but can never substitute it completely. Perfect sealing should be the plan of each clinical performance, in other words, leakage should be prevented.[1]

Glass ionomer cement (GIC) as a restorative material has several unique advantages. The content fluoride plays an important role with its cariostatic properties.[2] So far, GIC has proved to be the best restorative material in pediatric population compared with other restorative materials like amalgam or composites, however, GICs suffer from lower strength, wear resistance, and fracture toughness.[3] Reinforcement of GIC has, therefore, become a matter of increasing research efforts. In former experiments, various alumina, zirconia, silicon carbide, carbon fibers, or metal particles were used with encouraging results.[4]

Fiber reinforcement with reactive glass fibers is an attempt to achieve proper fiber embedding in the cement matrix. Short glass fiber reinforcement with a glass composition in the system $CaO–P_2O_5–SiO_2–Al_2O_3$ have been used to improve self-adhesion to human dentin.[3]

The concept of health has prevailed for centuries and is constantly evolving. Also, the dietary habits are changing with modernization. Data from national surveys indicate that calcium intakes of children are below the Recommended Dietary Allowance (RDA) and also high soft drink consumption might lead to excessive

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energy intake, which may contribute to childhood obesity and other health related problems.[5]

Commonly consumed soft drinks cause damage to the teeth due to their low pH and high titratable acidity leading to non-carious cervical tooth loss. The sugars in these drinks are metabolized by plaque micro-organisms to generate organic acids that bring about demineralization leading to dental caries.[6] These carbonated drinks and preserved fruit juices have led to an increased prevalence of dental erosion, as well as color changes of the restorations, thereby deteriorating the quality of the filling leading to its early replacement.[7] Real mixed fruit juice and Fanta were used for this study as they are readily available and children prefer colored beverages.

In vitro studies performed on primary teeth are limited and also incorporation of fibers into GIC for improved strength has been attempted by this study. Thus, the current study was conducted to compare and evaluate the microleakage of fiber-reinforced GIC and conventional glass ionomer cement restorations immersed in fruit drink, a fresh fruit juice and a soft drink and also to compare the effect of different immersion regimes (low, medium, and high) on the microleakage.

Materials and Methods

Study Design and Sample Size Calculation

This is an experimental *in vitro* study and the sample size was defined using unknown population size with a margin of error of 5% and by taking a confidence interval of 95%. A total of 8 teeth specimens were included in each of the subgroups based on the type of immersions. Therefore, a total of 24 teeth were included in each of the groups (Group I, Group II, Group III, and Group IV, based on the beverages used in the study). Hence, the sample size in the current study was estimated to be 96.

The sample size formula used is as follows:

$$n_1 = \frac{(\sigma_1^2 + \sigma_2^2 / K)(z_{1-\alpha/2} + z_{1-\beta})^2}{\Delta^2 In}$$

The notation for the formula is:

n_1 = Sample size of group I

n_2 = Sample size of group II

σ_1 = Standard deviation of group I

σ_2 = Standard deviation of group II

Δ = Difference in group means

K = Ratio = n_2/n_1

$z_{1-\alpha/2}$ = Two-sided z value (e.g., z = 1.96 for 95% confidence interval)

$z_{1-\beta}$ = Power

SD of microleakage in group I = 0.621

SD of microleakage in group II = 0.561

Δ = Difference in mean value = 2.20 – 1.38 = 0.81

K = 1

$$N = \frac{(0.621 \times 0.621 + 0.561 \times 0.561)\ (1.96 + 0.84)^2}{0.81 \times 0.81}$$

$$= 8.36 = 8$$

= 8 samples are needed in each subgroups

A sample size of 96 was obtained by taking 95% confidence interval and 5% margin of error.

The study was initiated after receiving permission from the Institutional Ethical Committee and was conducted in the year 2018–2019 and took 16–18 months for its completion.

Sample Collection and Initial Preparation

A total of 96 human exfoliating deciduous molars were selected. They were cleaned with water and the calculus, if any, was scaled using hand and ultrasonic scaler. They were then stored in deionized water to which 1% thymol was added.[8] This was done to disinfect the teeth.

Inclusion Criteria

- Exfoliated/extracted primary teeth (1st and 2nd maxillary and mandibular deciduous molars) with sound crown structure.

Exclusion Criteria

- Carious and previously restored primary 1st and 2nd primary molars.
- Teeth from patients or parents/guardians not willing to consent.

Standardized Class V cavity preparation (2 mm length, 2 mm width, and 1.5 mm depth) was prepared on the buccal and lingual surfaces of the teeth 1 mm above the Cementoenamel Junction (CEJ). The cavity preparation was standardized using William's graduated periodontal probe.

Manipulation of the Experimental Cement

Experimental fiber-reinforced glass ionomer cement (Exp-FRGIC) was prepared by adding discontinuous-glass fiber of 250–500 µm in length to the powder of GIC (GC Fuji II) in the weight ratio 10 wt% (Fig. 1). The length of fiber strand was manually cut in the required length. These fibers were manually mixed with the powder of GIC by thorough agitation of the mixture, to obtain a homogeneous powder. Then this homogeneous powder and the GIC liquid were mixed and manipulated strictly according to the manufacturer's guidelines.

The cavities on the buccal surface were restored with Exp-FRGIC while those on the lingual surface were restored with Type II Restorative GC (conventional GIC). The restorations were done in strict accordance with the manufacturer's guidelines and were stored in water at room temperature for 1 week. During this period, the teeth were subjected to 200 thermocycles between 5 and 55°C to simulate the oral environment.

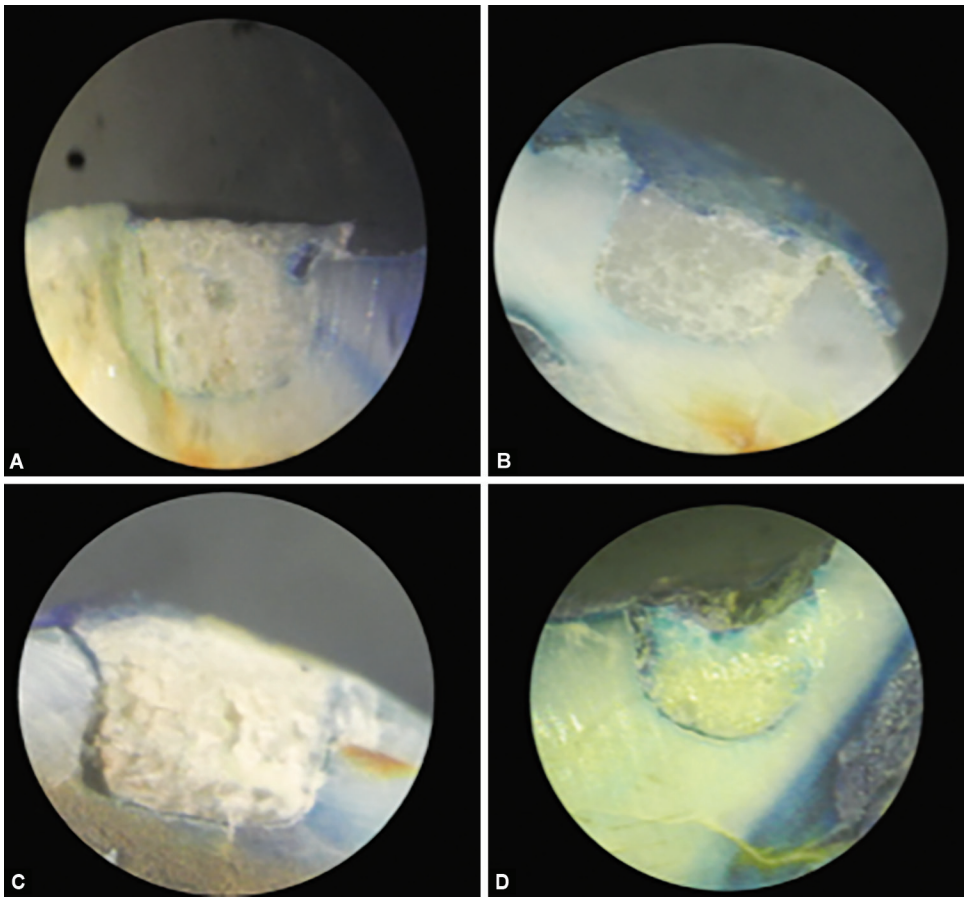
Sample Distribution

The restored 96 teeth were randomly divided into four groups. Each group consisted of 24 teeth. The division according to the test beverage was as follows:

- Group I – Fruit drink (Real mixed fruit juice – 24 teeth)
- Group II – Fresh fruit juice (Freshly prepared fruit juice – 24 teeth)*
- Group III – Soft drink (Fanta – 24 teeth)
- Group IV – Control group (Deionized water – 24 teeth)

*Fresh fruit juice was freshly prepared for the various immersion daily, using fruits like apple, orange, sweet lime, grapes, pineapple, pomegranate, and banana (commonly available in Indian markets and consumed by children). A quantity of 10–15 mL of each of them was used to prepare the mixed fruit juice but the proportion was not standardized to simulate the home-made preparation of these juices.

The samples were subjected to various immersion regimes given by Maupome et al., each immersion lasting for 5 min.[9]



Figs 1A to D: Microleakage scores (A) Score 0; (B) Score 1; (C) Score 2; (D) Score 3

Table 1: Sample distribution table

Group	*Low immersion (1 imm/day)*	*Medium immersion (5 imm/day)*	*High immersion (10 imm/day)*
Group I (Fruit drink)	8	8	8
Group II (Fresh fruit juice)	8	8	8
Group III (Soft drink)	8	8	8
Group IV (Control)	8	8	8

- Low immersion – 1 immersion/day
- Medium immersion – 5 immersions/day
- High immersion – 10 immersions/day

The sample groups were further divided into subgroups of 8 teeth each based on the immersion regime (Table 1).

The immersions were evenly distributed over a 12 hour period. The whole procedure was carried out for 8 days. Before and after each immersion, the samples were rinsed in 0.1 M phosphate buffered saline. When not immersed, they were stored in de-ionized water at room temperature. The teeth were then immersed in methylene blue dye for 24 hours at 37°C. The teeth were then thoroughly washed in water. Each tooth was sectioned into two halves buccolingually in an occluso-apical direction through the middle of each section was then observed under a Stereomicroscope (OLYMPUS) with a magnification of 40×. Microleakage of both halves was assessed.

Dye penetration was graded based on the extent of penetration along the occlusal wall of the restoration using the criteria recommended by Prabhakar et al.[10] [Fig. 2 (a-score 0), (b-score 1), (c-score 2), (d-score 3)].

- Score 0 – No dye penetration
- Score 1 – Dye penetration between the restoration and the tooth into enamel only
- Score 2 – Dye penetration between the restoration and tooth into enamel and dentin
- Score 3 – Dye penetration between restoration and tooth into the pulp

Intragroup comparison of Exp-FRGIC and conventional GIC according to immersion regimes were done separately using Chi-square test and at places where it could not be used Fisher's exact test was performed showing *p*-value (S - Significant $p < 0.05$, NS - Not significant $p > 0.05$).

Intergroup comparison of each cement Exp-FRGIC and conventional GIC in various immersion regimes was done separately for each immersion regime using multiple comparison by Tukey test.

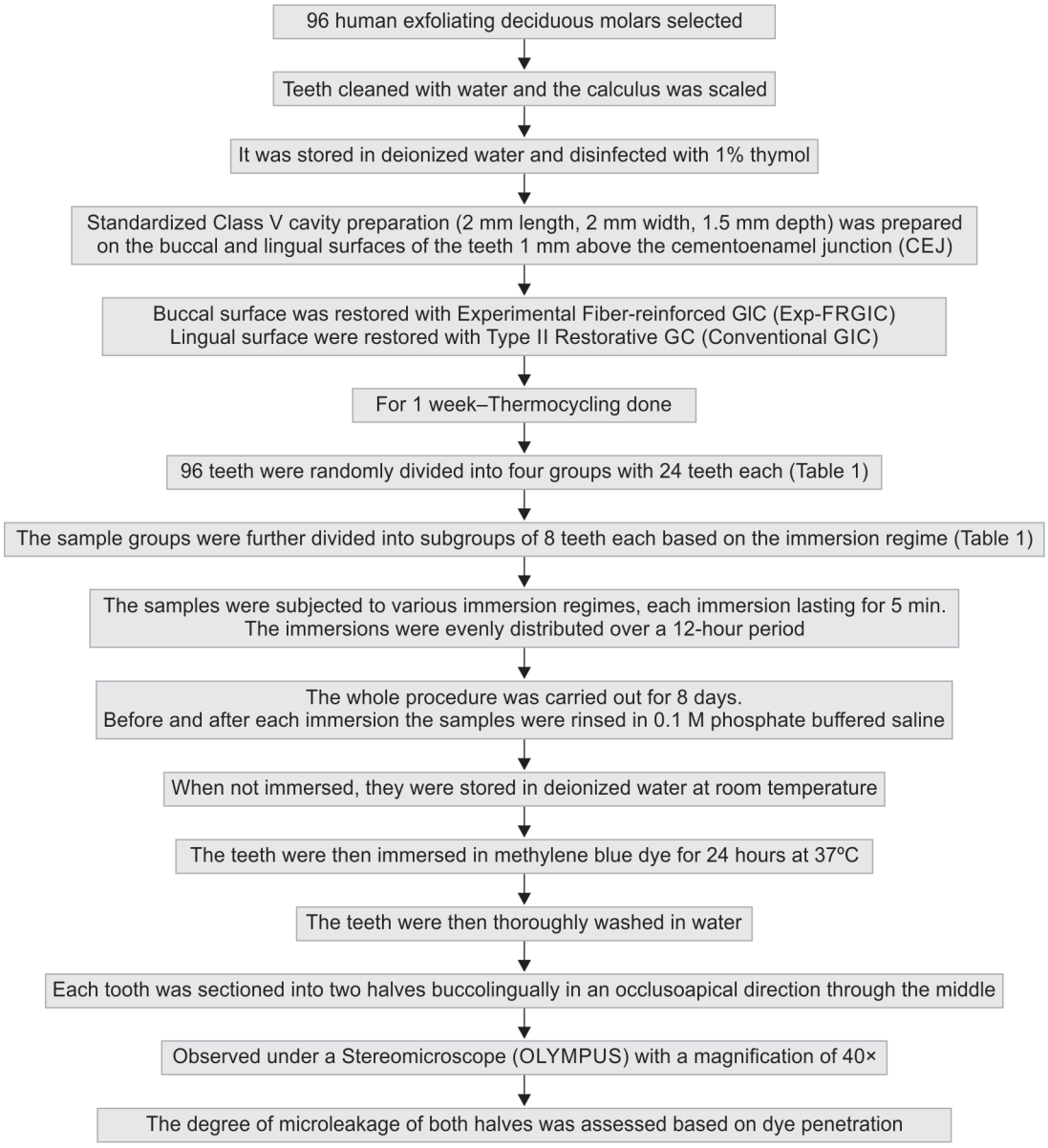


Fig. 2: Methodology flowchart

Statistical Analysis

Statistical analysis was done by using descriptive and inferential statistics using Kruskal–Wallis Chi-square test, Fisher's exact test, Mann–Whitney *U* test and multiple comparison: Tukey test. All of the statistical tests were run by the SPSS 22.0 version and GraphPad Prism 6.0 version software and $p < 0.05$ was considered as level of significance.

Results

The microleakage scores increased with increase in frequency of immersion for both Exp- FRGIC and conventional GIC in all the four groups (fruit drink, fresh fruit juice, and soft drink) meaning as the immersion period increased, more micro leakage occurred.

Intragroup comparison for Exp-Fiber-reinforced GIC, by using Chi-square test and at places where it could not be used, Fisher's exact test was performed showing *p*-value (S – Significant $p < 0.05$, NS – Not significant $p > 0.05$) showed significant microleakage when immersed in fresh fruit juice (Group II) ($p = 0.014$) at high immersion whereas intragroup comparison in conventional GIC, showed highest microleakage with soft drink (Group III) ($p = 0.002$) when compared with low and high immersion regimes (Tables 2 and 3).

Intergroup comparison between the various groups of Exp-Fiber-reinforced GIC and conventional GIC in low immersion using multiple comparisons using Tukey test did not depict any significant microleakage when immersed in any of the beverages (Tables 4a and b).

Intergroup comparison of Exp-Fiber-reinforced GIC at medium immersion regime showed more microleakage when immersed in soft drink (Group III) compared with fruit drink (Group I) ($p = 0.044$) and control (deionized water – Group IV) ($p = 0.044$) whereas intergroup comparison of conventional GIC at medium immersion regime showed maximum microleakage with specimen immersed

Table 2: Intragroup comparison of Exp-FRGIC according to immersion regimes

	Low vs Medium		*Low vs High*		*Medium vs High*	
Group	*χ^2-value*	*p-value*	*χ^2-value*	*p-value*	*χ^2-value*	*p-value*
Group I		1.00, NS	5.20	0.15, NS	4.28	0.23, NS
Group II	2.31	0.51, NS	8.50	0.014, S	7.28	0.06, NS
Group III	2.33	0.50, NS	9.90	0.019, NS	5.20	0.15, NS
Group IV		0.56, NS		0.56, NS		1.00, NS

*Chi-square test and Fisher's exact test, S, significant; NS, not significant

Table 3: Intragroup comparison for conventional GIC according to immersion regimes

	Low vs Medium		*Low vs High*		*Medium vs High*	
Group	*χ^2-value*	*p-value*	*χ^2-value*	*p-value*	*χ^2-value*	*p-value*
Group I	6.57	0.037, S	7.11	0.065, NS	2.66	0.44, NS
Group II	6.57	0.037, S	8.08	0.044, S	2.81	0.42, NS
Group III	9.80	0.020, S	14.00	0.002, S	2.82	0.42, NS
Group IV	4.60	0.10, NS	6.44	0.039, S	1.03	0.59, NS

*Chi-square test and Fisher's exact test, S, significant; NS, not significant

Table 4a: Intergroup comparison of Exp-FRGIC for low immersion in groups I, II, III and IV

				95% Confidence interval	
Group	*Mean difference (I–J)*	*Std. error*	*p-value*	*Lower bound*	*Upper bound*
Group I					
II	–0.125	0.342	0.983, NS	–1.059	0.809
III	–0.625	0.342	0.283, NS	–1.559	0.309
IV	0.125	0.342	0.983, NS	–0.809	1.059
Group II					
III	–0.500	0.342	0.474, NS	–1.434	0.434
IV	0.250	0.342	0.884, NS	–0.684	1.184
Group III					
IV	0.750	0.342	0.151, NS	–0.184	1.684

*Multiple comparisons: Tukey test

Table 4b: Intergroup comparison of conventional GIC for low immersion in groups I, II, III and IV

				95% Confidence interval	
Group	*Mean difference (I–J)*	*Std. error*	*p-value*	*Lower bound*	*Upper bound*
Group I					
II	0.000	0.240	1.000, NS	–0.657	0.657
III	–0.500	0.240	0.186, NS	–1.157	0.157
IV	0.000	0.240	1.000, NS	–0.657	0.657
Group II					
III	–0.500	0.240	0.186, NS	–1.157	0.157
IV	0.000	0.240	1.000, NS	–0.657	0.657
Group III					
IV	0.500	0.240	0.186, NS	–0.157	1.157

*Multiple comparisons: Tukey test

in soft drink (Group III) ($p = 0.044$) compared with deionized water (Group IV) ($p = 0.044$) followed by fruit drink (Group I) ($p = 0.014$), and fresh fruit juice (Group II) ($p = 0.014$) (Tables 5a and b).

Intergroup comparison of Exp-Fiber-reinforced GIC in high immersion regime, showed more microleakage with specimen immersed in soft drink (Group III) followed by fresh fruit juice (Group II), fruit drink (Group I) and deionized water (Group IV) whereas for conventional GIC in high immersion regime, showed maximum microleakage with soft drink (Group III) when compared with all other beverage used (Tables 6a and b).

Comparison of mean microleakage score at Exp-Fiber-reinforced GIC and conventional GIC in four groups at low, medium, and high immersion did not show any significant results statistically indicating no material was superior. However, maximum microleakage was observed in soft drink followed by fresh fruit juice and fruit drink.

Discussion

Pediatric restorative dentistry has evolved a long way from the age-old amalgam restorations to the present-day state-of-the-art nanomaterials with improved esthetics; but microleakage still remains a problem with the commonly used restorative materials.[11]

Glass ionomer cements till now are not considered as material of choice in the restoration of posterior teeth especially in high stress bearing areas.[12] The current study was proposed to improve the mechanical properties of conventional GIC that is already used in dental clinics as a restorative dental material. The reinforcing was

Table 5a: Intergroup comparison of Exp-FRGIC for medium immersion in groups I, II, III and IV

				95% Confidence interval	
Group	*Mean difference (I–J)*	*Std. error*	*p-value*	*Lower bound*	*Upper bound*
Group I					
II	−0.500	0.448	0.683, NS	−1.723	0.723
III	−1.250	0.448	0.044, S	−2.473	−0.026
IV	0.000	0.448	1.000, NS	−1.223	1.223
Group II					
III	−0.750	0.448	0.356, NS	−1.973	0.473
IV	0.500	0.448	0.683, NS	−0.723	1.723
Group III					
IV	1.250	0.448	0.044, S	0.026	2.473

*Multiple comparisons: Tukey test

Table 5b: Intergroup comparison of conventional GIC for medium immersion in groups I, II, III, and IV

				95% Confidence interval	
Group	*Mean difference (I–J)*	*Std. error*	*p-value*	*Lower bound*	*Upper bound*
Group I					
II	0.000	0.382	1.000, NS	−1.044	1.044
III	−1.250	0.382	0.014, S	−2.294	−0.206
IV	0.125	0.382	0.988, NS	−0.919	1.169
Group II					
III	−1.250	0.382	0.014, S	−2.294	−0.206
IV	0.125	0.382	0.988, NS	−0.919	1.169
Group III					
IV	1.375	0.382	0.006, S	0.331	2.419

*Multiple comparisons: Tukey test

Table 6a: Intergroup comparison of Exp-FRGIC for high immersion in groups I, II, III, and IV

				95% Confidence interval	
Group	*Mean difference (I–J)*	*Std. error*	*p-value*	*Lower bound*	*Upper bound*
Group I					
II	−0.125	0.33	0.982, NS	−1.04	0.79
III	−1.500	0.33	0.001, S	−2.42	−0.57
IV	0.875	0.33	0.067, NS	−0.04	1.79
Group II					
III	−1.375	0.33	0.002, S	−2.29	−0.45
IV	1.000	0.33	0.030, S	0.07	1.92
Group III					
IV	2.375	0.33	0.0001, S	1.45	3.29

*Multiple comparisons: Tukey test

Table 6b: Intergroup comparison of conventional GIC for high immersion in groups I, II, III, and IV

				95% Confidence interval	
Group	*Mean difference (I–J)*	*Std. error*	*p-value*	*Lower bound*	*Upper bound*
Group I					
II	0.125	0.439	0.992, NS	−1.07	1.32
III	−1.375	0.439	0.020, S	−2.57	−0.17
IV	0.625	0.439	0.496, NS	−0.57	1.82
Group II					
III	−1.500	0.439	0.010, S	−2.69	−0.30
IV	0.500	0.439	0.670, NS	−0.69	1.69
Group III					
IV	2.000	0.439	0.001, S	0.80	3.19

*Multiple comparisons: Tukey test

made by adding discontinuous glass fibers to GIC powder in the weight ratio of 10 wt%.[13]

The results of the current study showed generalized increase in microleakage scores with increase in frequency of immersion in all test beverages, for both experimental GIC and conventional GIC (Tables 2 and 3).

In Group I (Real fruit drink), conventional GIC showed statistically significant microleakage ($p = 0.037$) when low immersion regime was compared with medium immersion regime. In a similar study by Katge et al., nanoionomer and nanocomposite both showed statistically significant microleakage in medium and high immersion regimes, when compared with low immersion regime.[14] The comparison was not statistically significant between low and medium immersion regime.[14] Fruit drinks contain citric and ascorbic acids and also have stabilizing agents which maintains the pH of the drink which leads to demineralization and erosion.[15]

In Group II (fresh fruit juice), the microleakage scores increased as the frequency of immersion increased for both Experimental FRGIC and conventional GIC. This was in accordance with the study conducted by Maganur et al., in which the microleakage scores for Filtek™ flow and Vitremer™ increased with increase in frequency of immersion in Orange juice, and in a similar study by Katge et al.[7,14]

In Group III (Soft drink) also, the microleakage scores increased with increase in frequency of immersion for both Exp-FRGIC and Conv GIC. Similar to fresh fruit juice statistically significant difference in microleakage was noted only in conventional GIC group when low ($p = 0.002$) and medium ($p = 0.020$) immersion were compared with high immersion but no significant difference was observed in Exp-FRGIC. This is in accordance to the study conducted by Katge et al.[14] Similar study conducted by Maganur et al. in which the microleakage scores for Filtek™ flow and Vitremer™ increased with increase in frequency of immersion in cola drink group and a study conducted by Narsimha, comparing Dyract AP and Fuji II LC under various immersion regime in a cola drink, where statistically significant difference was observed between the two of all the three regimes.[7,16] This phenomenon could be attributed to the fact that carbonated cola beverages contain carbonic acid formed by the addition of carbon dioxide. Even when this carbon dioxide is blown off and the drinks have become 'flat' the pH remains low. In addition, carbonic acid is incorporated along with phosphoric acid in order to impart a tangy flavor to their drinks.[17]

The subgroup comparison of both the materials showed similar microleakage scores. Under different immersion regimes in test beverages, conventional GIC exhibited more microleakage than Experimental FRGIC in all groups. However, the result was not statistically significant. In the study conducted by Narsimha, Dyract AP (polyacid modified composite resin) and Fuji II L.C (resin modified glass ionomer restorative material) revealed similar microleakage scores.[16] In another study, Sabdi et al. concluded that Filtek Z250 has significantly lower degree of microleakage after acid exposure compared with conventional GIC, Resin modified GIC, and Silver Amalgam.[18]

Limitations

The limitations of the present study were:

- Even though method like thermocycling was performed to simulate the oral environment, it is difficult to obtain complete *in vivo* conditions when an *in vitro* study is performed. Steps like preservation in artificial saliva, simulation of occlusal or other mechanical force loading also needs to be performed.
- Dye penetration method can give only a 2D view of the amount of microleakage and that too only at the area where it is sectioned.
- The preservation time for the extracted teeth varied which may also influence its microleakage scores.

Future Directions

Increasing demand for tooth-colored esthetic restorative materials for permanent as well as for primary dentition has forced researchers to find alternatives to the currently available dental materials with enhanced esthetics, improved mechanics and clinical longevity and durability. Reinforcement of various fibers into the dental materials has always given rise to newer materials with increased strength and durability but efforts and further research needs to be conducted on the type of fibers incorporated, its length, ratio, and proportion, its form and biocompatibility. After a thorough investigation of all these aspects efforts should be made to introduce such materials commercially.

Fluid consumption patterns have changed with introduction of sweetened drinks and flavored milk in children as well as adults. An extensive research and development should be undertaken to alter the constituents in the modern-day fruit juice, soft drink, energy drink, and flavored milk to make them oral environment friendly and healthy by introducing remineralizing agents into them or addition of noncariogenic sweeteners.

Clinical Significance

Any restoration shows its full potential when the operator performs the technique intricately and follows manufacturer's guidelines. Superior GIC available in the market have proven benefits when compared with the conventional material. Also, the use of soft drinks and fruit juices should be limited in the diet of a growing child both to prevent caries and increase the longevity of a restoration.

Conclusion

It can be concluded from the study that the three beverages used in the study affected the microleakage of both restorative materials significantly. The microleakage scores increased in accordance with the frequency of the immersions. Also, soft drink caused the highest microleakage followed by fresh fruit juice and fruit drink. Conventional GIC exhibited more microleakage when compared with Exp-FRGIC in all the four groups; however, the comparison was not statistically significant. Newer studies incorporating fibers into GIC in various proportions need to be carried out and it can be commercially made available for restorative purposes. Also instead of fruit juices, whole fruits should be encouraged to be incorporated in the diet of a growing child.

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