

Comparative Evaluation of the Fluoride Releasing Ability and Microbial Attachment of Glass-Hybrid Restorative Material

MinKi Choi¹, Howon Park¹, Siyoung Lee², Haeni Kim¹, Juhyun Lee¹

¹Department of Pediatric Dentistry, Oral Science Research Center, College of Dentistry, Gangneung-Wonju National University, Gangneung, Republic of Korea

²Department of Microbiology and Immunology, College of Dentistry, Gangneung-Wonju National University, Gangneung, Republic of Korea

Abstract

This study aimed to compare the fluoride-releasing ability and degree of microbial attachment of a newly developed glass-hybrid restorative material (GH) with those of a high-viscosity glass ionomer (HvGIC), resin-modified glass ionomer (RMGI), and composite resin (CR). In addition, the correlation between fluoride-releasing ability and microbial attachment between materials was evaluated. Specimens were prepared in a disc shape and divided into 4 groups according to the materials (GH, HvGIC, RMGI, and CR). The fluoride release experiments were performed in each group (n = 15). The amount of fluoride released was measured on days 1, 3, 7, 14, 28, and 42 after storage. For the microbial attachment experiment, 12 specimens were produced per group using *Mutans Streptococci* (*S. mutans*), a cariogenic microorganism. *S. mutans* was cultured on the specimens for 24 hours, and the number of bacteria was measured. GH had the highest cumulative fluoride release and showed a significant difference when compared with RMGI ($p = 0.001$) and CR ($p < 0.0001$). Microbial attachment was the lowest in GH; however, no significant difference was observed between the materials ($p = 0.169$). There was no significant correlation between fluoride release from materials and microbial attachment ($p > 0.05$). From this perspective, remineralization of low-mineralized areas could be expected due to the high fluoride release of GH, and the effect of delaying the progression of dental caries could be predicted from the low cariogenic microbial attachment. Therefore, GH might be a useful restorative material for treating immature permanent teeth with hypomineralized enamel. However, further studies are needed about the degree of remineralization of hypomineralized areas after restoration and the capacity to recharge fluoride. [J Korean Acad Pediatr Dent 2024;51(2):132-139]

ORCID

MinKi Choi

<https://orcid.org/0009-0005-1832-2184>

Howon Park

<https://orcid.org/0000-0001-5741-9804>

Siyoung Lee

<https://orcid.org/0000-0001-8826-1413>

Haeni Kim

<https://orcid.org/0000-0002-4390-6270>

Juhyun Lee

<https://orcid.org/0000-0002-7586-9478>

Article history

Received July 27, 2023

Revised September 4, 2023

Accepted September 6, 2023

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Keywords

Glass-hybrid, Fluoride release, Microbial attachment

Corresponding author: Juhyun Lee

Department of Pediatric Dentistry, College of Dentistry, Gangneung-Wonju National University, 7 Jukheon-gil, Gangneung, 25457, Republic of Korea

Tel: +82-33-640-2452 / Fax: +82-33-640-3113 / E-mail: ljh55@gwnu.ac.kr

Funding information

This work was supported by the Scientific Research (SR2303) of Gangneung-Wonju National University Dental Hospital.

Introduction

Enamel hypomineralized teeth have enamel defects accompanied by altered translucency, reduced enamel hardness, and low mineral content[1]. Aprismatic layers in the enamel might speed up the growth of caries. Due to the wider interprismatic spaces that make it easier for microorganisms to spread into the dentin and adversely affect the pulp[2]. Because of this structural difference, the possibility of pulp exposure during treatment is higher than that of normal teeth. To reduce this possibility, clinicians consider remineralizing the tooth by temporal restoration with fluoride-releasing material after selective tooth preparation[3,4].

In addition to the restorative material, one thing to consider is the plaque deposition. Whether the tooth has a restored surface or not, biofilm accumulation is a necessary factor for caries progression[5], and it is widely known that *Mutans Streptococci* (*S.mutans*) contribute significantly to the development of caries[6]. *S.mutans* synthesizes glucan from sugar to induce bacterial biofilm attachment, and long-term biofilm formation on the surface affects the progression of dental caries, including secondary caries[7].

Considering the above two conditions, the most commonly used material in pediatric dentistry is the glass ionomer series. One of the characteristics of glass ionomers is that they release fluoride. Fluoride reduces tooth demineralization, induces remineralization, and hinders

caries formation[7]. The higher the fluoride content in the restoration, the better it is, if it does not interfere with the physical properties of the restoration[8]. Glass ionomer was developed as the first fluoride-releasing restorative material, but it has poor physical properties. Therefore, attempts to improve the physical properties of glass ionomers have been going on for decades.

Recently, a manufacturer developed a glass-hybrid (GH) restorative material by adding ultra-fine, highly reactive, fine glass nanoparticles to a high-viscosity glass ionomer and introduced it as a material that restores the stress-bearing area of enamel hypomineralized teeth. Previous studies have addressed physical properties of GH such as compressive strength, flexural strength, surface roughness, and microleakage[9-11]. To the best of our knowledge, studies on fluoride release and the degree of attachment by cariogenic microorganisms are lacking.

Therefore, the purpose of this study is to compare the fluoride-releasing ability and degree of attachment of cariogenic microorganisms to GH with high-viscosity glass ionomer (HvGIC), resin-modified glass ionomer (RMGI), and composite resin (CR).

Materials and Methods

1. Research materials

GH, HvGIC, RMGI, and CR were used for this study. Materials used in the study are listed in Table 1.

Table 1. Materials used in this study and grouping

Materials (Lot number)	Group	Product	Composition	Manufacturer
Glass-Hybrid Material (2106052)	GH	Equia Forte Ht fil (A2 shade)	Liquid: Polyacrylic acid, water, polybasic carboxylic acid Powder: Fluoro-aluminosilicate glass	GC, Tokyo, Japan
High Viscosity Glass Ionomer (2207141)	HvGIC	Fuji IX GP Extra (A2 shade)	Liquid: Polyacrylic acid, water, polybasic carboxylic acid Powder: Fluoro-aluminosilicate glass	GC, Tokyo, Japan
Resin modified Glass Ionomer (2208231)	RMGI	Fuji II LC Capsule (A2 shade)	Liquid: Water, Polyacrylic acid, HEMA, UDMA, Camphoroquinone Powder: Fluoro-aluminosilicate glass, 5% polyacrylic acid	GC, Tokyo, Japan
Composite Resin (NE83494)	CR	Filtek Z350XT flowable (A2 shade)	Monomer: Bis-GMA, UDMA, TEDGDMA, Bis-EMA Filler: Silica filler, Zirconia filler, Aggregated zirconia/silica cluster filler	3M ESPE, St. Paul, MN, USA

HEMA: Hydroxyethyl methacrylate; UDMA: Urethane dimethacrylate; Bis-GMA: Bisphenol A-diglycidyl methacrylate; TEDGDMA: Triethylene glycol dimethacrylate; Bis-EMA: Bisphenol A-diglycidyl methacrylate ethoxylated.

2. Research method

1) Specimen preparation

Two metal molds with a diameter of 7.0 mm and a height 2.0 mm were used. The substance was poured into the metal mold, which had a mylar strip sandwiched between the bottom and the glass plate. It was pressed with another mylar strip on top to make a flat surface. For the RMGI and CR specimens, light curing was performed on each surface for 20 s. The photopolymerizer used was Valo (Ultradent Products Inc., South Jordan, UT, USA), which is an LED polymerizer in a high-power mode (1400 mW/cm²).

2) Measurement of fluoride release

Fifteen specimens per group were used for measuring the fluoride release. Each specimen was put into a polyethylene tube with 2.0 ml of sterile distilled water, sealed, and kept in a constant-temperature (37°C) water bath.

Fluoride was measured on days 1, 3, 7, 14, 28, and 42. After measurement, sterile distilled water was replaced. The amount of fluoride release was measured after correction of concentration using 1, 10, and 100 ppm fluoride standard solutions (Fluoride with TISAB II Standard, Thermo Scientific™ Orion™, Beverly, MA, USA) at each measurement, and a pH/ISE meter (920A+, Thermo Scientific™ Orion™, Beverly, MA, USA) was used to measure the fluoride released in each solution.

3) Bacterial culture

S. mutans ATCC 25175 was added to the brain heart infusion medium (BHI broth; Becton, Dickinson and Company, Sparks, MD, USA) and cultured for 18 h at 37°C with 5% CO₂. Bacterial turbidity was measured using a spectrophotometer (Smart Plus 2700; Young-woo instrument, Seoul, Korea). The culture was diluted to 1.0×10^9 colony-forming units (CFU)/mL for measurements.

4) Formation of a cariogenic microbial film

Twelve specimens per group were used for the microbial attachment. Cylindrical specimens were sealed

with silicon impression material (I-SiL Poly Vinyl Siloxane Impression Material, Spident, Incheon, Korea). The specimens were cultured in 1980 µL of BHI broth supplemented with 1% sucrose and 20 µL of *S. mutans*. Therefore, the final concentration of bacteria was set to 1.0×10^7 CFU/mL. The specimens were then stored in a 5% CO₂ incubator for 24 h.

5) Bacterial count

After each specimen was rinsed twice with phosphate-buffered saline (PBS), a bacterial culture solution was obtained by sonication (VC 100; Sonics & Materials Inc., Danbury, CT, USA) for 20 s. After sonication, 100 µL of the culture medium was distributed on a blood agar plate and diluted to 1/1000 with PBS. The plate was then incubated for 72 h at 37°C with 5% CO₂. The bacterial colonies were counted using the naked eye, and the final number of bacteria was analyzed by conversion to a log scale.

6) Statistical analysis

All statistical analyses were performed using the SPSS software version 25.0 (SPSS Corp., Armonk, NY, USA). The statistical significance was verified for fluoride release and microbial attachment in each group using the Kruskal-Wallis test. The Mann-Whitney test was performed as a post-hoc analysis, and the resulting values were corrected using the Bonferroni correction method. The Pearson's correlation analysis was used to confirm the relationship between fluoride release and microbial attachment.

Results

1. Fluoride release

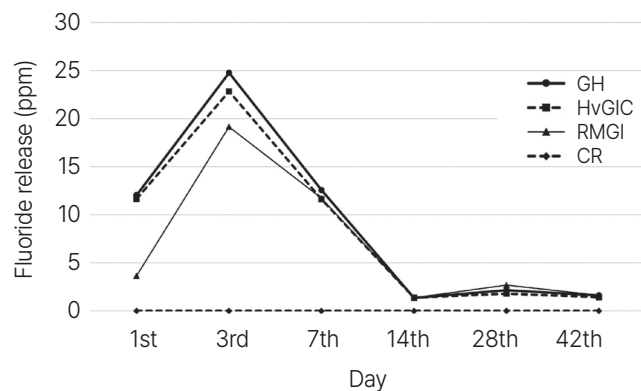
Table 2 and Fig. 1. show the amount of released fluoride per day. Fluoride release was observed in all groups except the CR group. The amount of fluoride released was the highest on the 3rd day of measurement and showed a decreasing trend over time.

The accumulated fluoride release is shown in Table 3 and Fig. 2. The amount of fluoride released over 42 days

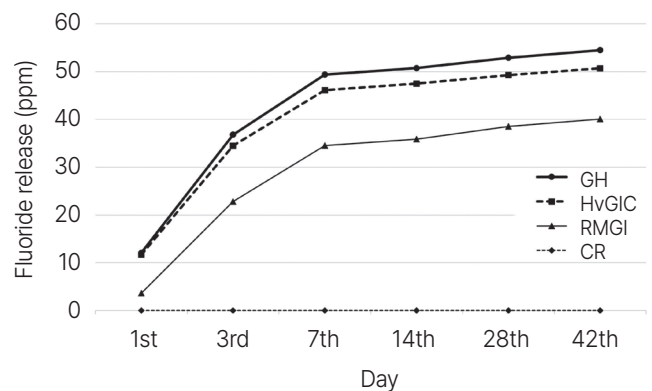
Table 2. Fluoride release by date in each group

Group	Fluoride release (Mean \pm SD, ppm)					
	1 st day	3 rd day	7 th day	14 th day	28 th day	42 th day
GH	12.05 \pm 2.16	24.76 \pm 5.42	12.57 \pm 0.53	1.35 \pm 0.07	2.15 \pm 0.26	1.61 \pm 0.82
HvGIC	11.66 \pm 2.700	22.82 \pm 6.34	11.62 \pm 1.12	1.38 \pm 0.05	1.79 \pm 0.64	1.41 \pm 0.74
RMGI	3.64 \pm 2.12	19.15 \pm 1.85	11.73 \pm 0.191	1.352 \pm 0.02	2.67 \pm 0.25	1.55 \pm 0.64
CR	0	0	0	0	0	0

GH: Glass hybrid; HvGIC: High-viscosity glass Ionomer; RMGI: Resin-modified glass ionomer; CR: Composite resin.

**Fig. 1.** Fluoride release by date in each group.

GH: Glass Hybrid; HvGIC: High-viscosity glass Ionomer; RMGI: Resin-modified glass ionomer; CR: Composite resin.

**Fig. 2.** Accumulated fluoride release in each group for 42 days. GH: Glass Hybrid; HvGIC: High-viscosity glass Ionomer; RMGI: Resin-modified glass ionomer; CR: Composite resin.**Table 3.** Accumulated fluoride release in each group for 42 days

Group	Cumulative Fluoride Release (Mean \pm SD, ppm)	<i>p</i> -value
GH	54.49 \pm 5.93 ^a	< 0.0001
HvGIC	50.68 \pm 8.77 ^{ab}	
RMGI	40.16 \pm 2.87 ^b	
CR	0 ^c	

p value from Kruskal-Wallis test.

a, b, c: The same superscript letters in the columns indicate that they are not significantly different by the Mann-Whitney test and Bonferroni correction method.

GH: Glass Hybrid; HvGIC: High-viscosity glass Ionomer; RMGI: Resin-modified glass ionomer; CR: Composite resin.

was the highest for GH, followed by HvGIC, RMGI, and CR. GH showed significantly higher fluoride release than RMGI ($p = 0.001$) and CR ($p < 0.0001$), and no significant difference was observed between GH and HvGIC ($p = 0.929$).

2. Microbial (*S. mutans*) attachment

A comparison of the degree of microbial attachment between the groups showed the highest ranking in the order of CR, HvGIC, RMGI, and GH, and no significant differences were observed between the materials ($p = 0.169$) (Table 4, Fig. 3).

Table 4. Microbial attachment according to materials

Group	Microbial attachment (Mean ± SD)	<i>p</i> value
GH	5.30 ± 0.14	0.169
HvGIC	5.31 ± 0.15	
RMGI	5.37 ± 0.13	
CR	5.45 ± 0.20	

p value from Kruskal–Wallis test.

GH: Glass Hybrid; HvGIC: High-viscosity glass Ionomer; RMGI: Resin-modified glass ionomer; CR: Composite resin.

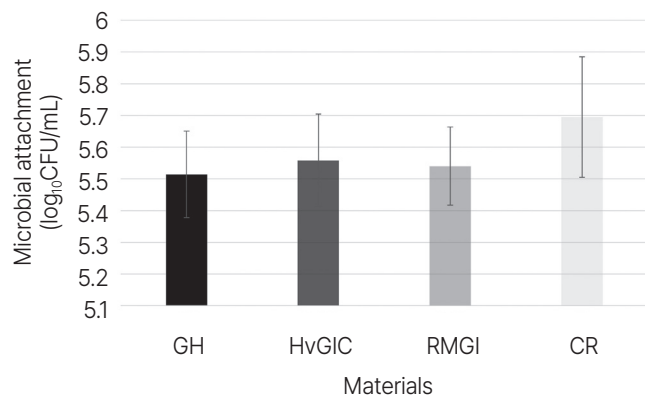


Fig. 3. Microbial attachment according to materials.

GH: Glass Hybrid; HvGIC: High-viscosity glass Ionomer; RMGI: Resin-modified glass ionomer; CR: Composite resin.

3. Correlation between fluoride release and microbial attachment

Analysis of the correlation between the degree of fluoride release and microbial attachment between the groups revealed no significant relationship ($p > 0.05$) (Table 5).

Table 5. Correlation between fluoride release and microbial attachment

	GH (Microorganism)	HvGIC (Microorganism)	RMGI (Microorganism)	Resin (Microorganism)
GH (Fluoride)	0.943			
HvGIC (Fluoride)		0.83		
RMGI (Fluoride)			0.34	
Resin (Fluoride)				.

p value from Pearson’s correlation analysis.

GH: Glass Hybrid; HvGIC: High-viscosity glass Ionomer; RMGI: Resin-modified glass ionomer; CR: Composite resin.

Discussion

Fluoride reduces demineralization, increases remineralization, inhibits microorganisms and biofilm formation, and inhibits bacterial growth[12]. Ibraheem and Al-Qutib[13] reported that when a fluoride-containing restoration was placed on a tooth, a radiopaque region was formed along the interface between the dentin and the restoration to suppress the potential for secondary caries formation around the restoration, and that the GI restoration had a stronger effect. Hicks et al.[14] stated that carious lesions adjacent to fluoride-releasing restorative materials exhibit a remineralization effect. Hahnel et al.[15] claimed that the release of fluoride in the restoration contributes to controlling microbial formation at an early stage. For this reason, it would be a reasonable choice for pediatric dentists to consider fluoride-releasing restorative materials as temporary fillings when treating hypomineralized enamel.

The GH used in this study was developed from a high-viscosity glass ionomer. According to the manufacturer, the physical properties and maneuverability of the material were improved by adding a high molecular weight polyacrylic acid and ultra-fine highly reactive glass nanoparticles to existing ingredients[16]. In a clinical study, GH was found to be effective in a 12-month evaluation when treating teeth affected by MIH[17].

In this study, the fluoride release experimental period was set to 42 days, based on the results of previous studies. Dijkman and Arends[18] compared the gap between restorations and enamel among fluoride-containing and non-fluoride restorations and reported that the de-

degree of demineralization and mineral loss significantly decreased after approximately 1 month. Therefore, it was confirmed that at least 28 days should be set as the experimental period to evaluate the degree of fluoride release over a short period of time.

Regarding the amount of fluoride released by the date of glass ionomer-based materials, all the materials showed the highest amount of fluoride release on the third day and a decreasing tendency thereafter. Wiegand et al.[19] stated that a large amount of fluoride release during the first 24 h is caused by rapid release from the surface of glass particles reacting with polyalkenoate acid during the glass ionomer setting reaction. Bell et al.[20] found that the fluoride content of a specimen with a diameter of 6.0 mm and a thickness of 1.5 mm that was submerged in artificial saliva was 1.0 ppm after 10 min, and quickly rose to 15 ppm after 24 h. Yap et al.[21] reported a high fluoride release for up to 5 days in an *in vitro* study, followed by a decreasing pattern. Compared with various experimental studies, this study also followed the fluoride-releasing tendencies of existing studies. The initial rapid release of fluoride was attributed to the rapid dissolution on the outer surface as a short-term reaction. Subsequently, the fluoride release gradually decreased, and a low level (plateau) of fluoride was continuously released. This is because of the ability of continuous fluoride release to occur through the pores in the materials[22-24].

In terms of accumulated fluoride release, the GH group released more fluoride than HvGIC. However, the difference was not significant. Although the basic components of the two materials are similar, the difference in the amount of fluoride released is thought to be due to the large surface area that can react with the inclusion of highly reactive nanoparticles between the large particles[24].

Previous studies comparing fluoride release from conventional glass ionomers and RMGIs have reported conflicting results. Zebić et al.[25] reported that RMGI showed a lower fluoride release than that by conventional glass ionomers. According to Momoi and McCabe[26], when comparing the fluoride release of RMGI and con-

ventional glass ionomer, there was no discernible difference between the groups and they had the capacity to emit comparable levels of fluoride. In this study, the GH group showed a significantly higher fluoride release than RMGI. We speculate that the polymer entangled with the polyalkenoate chain in the photopolymerized glass ionomer hinders fluoride release.

According to the results of the microbial attachment test, GH showed the least microbial attachment, followed by RMGI, HvGIC, and CR in order, with no obvious distinctions between the materials. In this study, since GH showed a significantly higher release in terms of the quantity of the fluoride release, it was expected that a significant difference would appear in the degree of microbial attachment, but the actual result was different from the expectation. DeSchepper et al.[27] and Herrera et al.[28] reported that fluoride's impact on bacterial cells is influenced by both its concentration and the pH at which microorganisms attach. In our view, as mentioned above, fluoride inhibits the formation of microorganisms and biofilms, but in addition to the amount of fluoride released, it is estimated that the surface properties of the restorative material affect the microbial attachment. Smoother surfaces are known to accumulate less plaque, and there is evidence linking surface roughness and bacterial adherence[29]. Since the design of this study did not include the process of polishing the specimens, it is expected that the surface characteristics of the material itself, such as surface roughness, hydrophilicity or hydrophobicity, surface chemistry between materials, and bacterial strain, might influence the results[30]. Therefore, it seemed that no significant correlation was found between the fluoride release and the microbial attachment.

Summarizing this study, GH showed significantly higher fluoride release and, although not significantly, less microbial attachment compared to other materials. In light of this, a remineralization effect could be expected in the hypomineralized areas through the release of fluoride from the GH. In addition, the effect of slowing down the progress of caries could be estimated through the attachment of a few microorganisms.

The limitation of this study was that it was a laboratory study; therefore, it did not reflect the actual oral environment or clinical circumstances and was a short-term study. Another limitation was that a qualitative evaluation of the surface roughness of dental restorative materials, which can have an important effect on microbial attachment, was not performed.

This study is valuable because there are only a few studies related to microbial attachment, although there are several studies on comparisons of the physical properties of restorative materials. In the future, additional studies comparing the fluoride recharge capacity or remineralization with existing glass ionomer-based materials are needed for clinical use.

Conclusion

In this study, GH showed a significantly higher fluoride release than RMGI and CR. The microbial attachment was also the lowest in the GH group. This difference, though, was insignificant. The level of fluoride release and microbial attachment did not significantly correlate with one another. When treating enamel hypomineralized teeth, remineralization could be expected due to the high fluoride release of GH, and the effect of delaying the progression of dental caries could be predicted from the low cariogenic microbial attachment. However, further studies are needed concerning the capacity of fluoride recharge and remineralization.

Conflict of Interest

The authors have no potential conflicts of interest to disclose.

Acknowledgments

This work was supported by the Scientific Research (SR2303) of Gangneung-Wonju National University Dental Hospital.

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