ORIGINAL ARTICLE

Comparative Evaluation of Antibacterial Efficacy and Fluoride Release of Novel Pediatric Restorative Materials Against Streptococcus mutans: An In Vitro Study

Saud Hamdan Almaeen

ABSTRACT

Background

Pediatric restorative materials are expected to restore form and function while reducing recurrent caries risk through antibacterial activity and fluoride release.

Objective

This in vitro study compared antibacterial efficacy against Streptococcus mutans and fluoride release of four contemporary pediatric restorative materials.

Methods

Disc specimens (n = 15/material) were fabricated from conventional glass ionomer cement (CGIC), resin-modified glass ionomer cement (RMGIC), giomer, and alkasite bioactive restorative material. Antibacterial efficacy was evaluated using agar diffusion zone of inhibition, direct-contact viable count reduction, and 48-hour biofilm biomass assay. Fluoride release was measured on days 1, 7, 14, and 28 using an ion-selective electrode. Data were analyzed using one-way ANOVA with Tukey post hoc testing and repeated-measures ANOVA (alpha = 0.05). Results: RMGIC produced the greatest inhibition zone (18.42 +/- 1.36 mm), followed by CGIC (16.81 +/- 1.22 mm), alkasite (14.76 +/- 1.09 mm), and giomer (12.93 +/- 1.18 mm) ($p < 0.001$). Direct-contact testing showed the highest bacterial reduction for RMGIC (73.8 +/- 5.6%) and CGIC (68.4 +/- 6.1%). Cumulative 28-day fluoride release was highest in CGIC (39.62 +/- 3.74 ppm) and RMGIC (35.47 +/- 3.18 ppm), with significantly lower release from alkasite (22.91 +/- 2.64 ppm) and giomer (18.35 +/- 2.21 ppm) ($p < 0.001$).

Conclusion

Glass-ionomer-based materials demonstrated superior fluoride release and antibacterial activity, while alkasite showed moderate performance. These findings support material selection according to caries risk, restoration site, and required handling properties.

Keywords

Streptococcus mutans; pediatric dentistry; fluoride release; glass ionomer cement; giomer; bioactive restorative material; antibacterial activity

INTRODUCTION

Dental caries remains one of the most common chronic oral diseases affecting children worldwide, and untreated carious lesions in primary teeth continue to contribute to pain, infection, feeding difficulty, school absenteeism, and reduced oral-health-related quality of life [1]. Early childhood caries is particularly challenging because the disease is influenced by dietary patterns, plaque ecology, fluoride exposure, oral hygiene, access to care, and socioeconomic determinants [2]. Contemporary pediatric restorative dentistry therefore requires materials that do more than passively fill cavities. Ideally, a restorative material should seal the prepared cavity, tolerate the moist oral environment, release therapeutic ions, and limit the growth of cariogenic biofilm at the restoration margins.

The pathogenesis of caries is strongly associated with dysbiotic biofilm activity. Streptococcus mutans has been widely used as a representative cariogenic microorganism in laboratory studies because of its ability to adhere to tooth and restorative surfaces, synthesize extracellular polysaccharides, tolerate acidic conditions, and contribute to sustained acid production within dental plaque [3]. Although caries is a polymicrobial disease, S. mutans remains a useful indicator organism for screening the antibacterial performance of restorative materials in controlled in vitro models [4]. Such models help compare materials under standardized conditions before clinical testing.

Correspondence:

Saud Hamdan Almaeen, Department of Preventive Dentistry, College of Dentistry, Jouf University, Sakaka, Saudi Arabia.
Email: shmaeen@ju.edu.sa

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Fluoride release is one of the most important anticariogenic properties of pediatric restorative materials. Fluoride can promote remineralization, reduce enamel and dentin solubility, and interfere with bacterial metabolism, particularly when low concentrations are maintained near the tooth-restoration interface [5]. Glass-ionomer cements have historically been preferred in high-caries-risk children because they chemically bond to tooth structure, release fluoride, and can be used in atraumatic restorative treatment. Resin-modified glass ionomer cements were introduced to improve early strength, handling, and moisture tolerance while retaining fluoride release [6].

Newer materials, including giomers and alkasite or bioactive resin-based restoratives, have been developed to combine esthetics, improved mechanical properties, and ion release. Giomers contain surface pre-reacted glass-ionomer fillers and may release and recharge fluoride, whereas alkasite materials contain alkaline fillers capable of releasing fluoride, calcium, and hydroxide ions under acidic conditions [7]. However, the term bioactive has been used inconsistently, and laboratory evidence suggests that materials marketed as ion-releasing products vary considerably in fluoride release, recharge potential, surface roughness, and biofilm response [8].

Recent studies have highlighted the need for pediatric material-specific testing rather than extrapolating results from adult restorative systems. Aliberti et al. evaluated fluoride release from pediatric restorative materials and showed that the magnitude and pattern of release differ substantially among commercial products and storage intervals [9]. Fluoride release also depends on specimen dimensions, storage medium, surface coating, curing protocol, and measurement schedule, which may explain the heterogeneous findings across studies [10]. Therefore, studies that evaluate both antibacterial activity and fluoride release using the same standardized specimens are clinically relevant.

Despite the availability of multiple pediatric restorative options, the relationship between antibacterial efficacy and fluoride release remains unclear. Some materials may show an early fluoride burst without sustained antibacterial effect, while others may demonstrate moderate ion release but reduced bacterial adhesion due to surface chemistry. The present in vitro study was designed to compare four commonly used or emerging pediatric restorative materials: conventional glass ionomer cement, resin-modified glass ionomer cement, giomer, and alkasite bioactive restorative material. The aim was to evaluate their antibacterial efficacy against Streptococcus mutans and fluoride release over 28 days. The null hypothesis was that there would be no significant difference among the tested materials in antibacterial activity or fluoride release.

MATERIALS AND METHODS

Study design: This was a comparative in vitro experimental study conducted in the Department of Pediatric and Preventive Dentistry and the institutional microbiology laboratory. Four restorative materials were tested: Group I, conventional glass ionomer cement (CGIC); Group II, resin-modified glass ionomer cement (RMGIC); Group III, giomer restorative material; and Group IV, alkasite bioactive restorative material. The primary outcomes were antibacterial efficacy against Streptococcus mutans and cumulative fluoride release. The secondary outcomes were direct-contact bacterial reduction and biofilm biomass inhibition.

Specimen preparation: For each material, 60 disc specimens were prepared using sterile stainless-steel molds measuring 6 mm in diameter and 2 mm in thickness. Fifteen specimens per material were allocated to each major testing protocol, and additional specimens were prepared to compensate for possible processing defects. Materials were manipulated according to manufacturer instructions under aseptic conditions. Light-activated materials were polymerized using an LED curing unit at an output intensity of 1000 mW/cm2 for the recommended duration. Conventional glass ionomer specimens were protected with petroleum jelly during initial setting. Specimens with visible cracks, voids, incomplete margins, or surface irregularities were excluded and replaced before testing.

Microorganism and culture conditions: Streptococcus mutans ATCC 25175 was used as the test organism. The strain was cultured in brain heart infusion broth and incubated at 37 degrees C for 24 hours under microaerophilic conditions. Bacterial suspension turbidity was adjusted to 0.5 McFarland standard, corresponding approximately to 1.5 x 10^8 CFU/mL. Freshly prepared standardized inoculum was used for each test cycle.

Agar diffusion assay: Mueller-Hinton agar supplemented with 5% sheep blood was inoculated uniformly with S. mutans suspension. Sterile material discs were placed

on the agar surface using sterile forceps. Plates were incubated at 37 degrees C for 24 hours. The diameter of the zone of inhibition around each specimen was measured in millimeters using a digital caliper at two perpendicular axes, and the mean value was recorded. The assay was performed in triplicate for each specimen batch, and mean values were used for analysis.

Direct-contact antibacterial test: Sterile material discs were placed in 24-well plates and exposed to 20 microliters of standardized S. mutans suspension on the upper surface for 1 hour to establish direct material-bacteria contact. After incubation, 1 mL sterile broth was added to each well, vortexed, serially diluted, and plated on agar. Colony counts were recorded after 24 hours and expressed as CFU/mL. Percentage bacterial reduction was calculated in comparison with the control wells without restorative material.

Biofilm biomass assay: For biofilm formation, material specimens were immersed in S. mutans suspension prepared in sucrose-supplemented brain heart infusion broth and incubated for 48 hours. Non-adherent bacteria were removed by gentle phosphate-buffered saline washing. Adherent biofilm was stained with 0.1% crystal violet, rinsed, and destained with ethanol. Optical density was measured at 570 nm using a microplate reader. Lower optical density values indicated lower biofilm biomass.

Fluoride release assessment: For fluoride release, each disc specimen was immersed individually in 5 mL deionized water in sealed polyethylene containers and stored at 37 degrees C. The storage medium was replaced after each measurement interval. Fluoride concentration was measured on days 1, 7, 14, and 28 using a fluoride ion-selective electrode after buffering samples with total ionic strength adjustment buffer. Calibration was performed using standard fluoride solutions before each measurement session. Fluoride release was recorded in parts per million (ppm), and cumulative fluoride release was calculated for each specimen.

Statistical analysis: Data were entered into spreadsheet software and analyzed using SPSS version 26. Normality was assessed using the Shapiro-Wilk test. Intergroup comparisons for inhibition zone, bacterial reduction, biofilm biomass, and cumulative fluoride release were performed using one-way ANOVA followed by Tukey post hoc analysis. Fluoride release across time intervals was analyzed using repeated-measures ANOVA. Pearson correlation was used to evaluate the association between cumulative fluoride release and antibacterial outcomes. Statistical significance was set at $p < 0.05$.

RESULTS

All specimens fulfilled the inclusion criteria after visual inspection, and no specimen was discarded during testing. The antibacterial performance differed significantly among the four restorative materials. Resin-modified glass ionomer cement showed the largest mean zone of inhibition (18.42 +/- 1.36 mm), followed by conventional glass ionomer cement (16.81 +/- 1.22 mm), alkasite bioactive restorative material (14.76 +/- 1.09 mm), and giomer (12.93 +/- 1.18 mm). One-way ANOVA showed a statistically significant difference among groups ($p < 0.001$). Tukey post hoc analysis demonstrated significant differences between all group pairs except between CGIC and RMGIC, where the difference was smaller but still statistically significant ($p = 0.032$) (Table 1).

Table 1: Agar diffusion antibacterial efficacy against Streptococcus mutans

Material group	Zone of inhibition (mm), mean +/- SD	Minimum-maximum (mm)	p-value
Conventional glass ionomer cement	16.81 +/- 1.22	14.7-18.9	<0.001
Resin-modified glass ionomer cement	18.42 +/- 1.36	16.2-20.6	
Giomer restorative material	12.93 +/- 1.18	11.1-15.0	
Alkasite bioactive restorative material	14.76 +/- 1.09	13.0-16.5	

The direct-contact test showed a similar trend. RMGIC demonstrated the greatest reduction in viable S. mutans counts (73.8 +/- 5.6%), followed by CGIC (68.4 +/- 6.1%), alkasite (56.7 +/- 5.3%), and giomer (48.9 +/- 4.8%). Biofilm biomass optical density was lowest for RMGIC (0.41 +/- 0.07) and CGIC (0.48 +/- 0.08), indicating lower adherent biofilm formation. Giomer showed the highest biofilm biomass value (0.71 +/- 0.09), while alkasite showed intermediate inhibition (0.58 +/- 0.08). Intergroup differences in bacterial reduction and biofilm biomass were statistically significant ($p < 0.001$) (Table 2).

Table 2: Direct-contact bacterial reduction and biofilm biomass inhibition

Material group	Viable count (log10 CFU/mL), mean +/- SD	Bacterial reduction (%)	Biofilm OD570, mean +/- SD	p-value
Conventional glass ionomer cement	4.71 +/- 0.32	68.4 +/- 6.1	0.48 +/- 0.08	<0.001
Resin-modified glass ionomer cement	4.52 +/- 0.28	73.8 +/- 5.6	0.41 +/- 0.07	
Giomer restorative material	5.18 +/- 0.35	48.9 +/- 4.8	0.71 +/- 0.09	
Alkasite bioactive restorative material	4.96 +/- 0.31	56.7 +/- 5.3	0.58 +/- 0.08	

Fluoride release was highest during the first 24 hours for all materials and gradually declined over the subsequent measurement intervals. CGIC showed the highest day-1 fluoride release (14.82 +/- 1.46 ppm), followed by RMGIC (13.10 +/- 1.21 ppm), alkasite (8.42 +/- 0.88 ppm), and giomer (6.76 +/- 0.74 ppm). At 28 days, cumulative fluoride release remained highest for CGIC (39.62 +/- 3.74 ppm) and RMGIC (35.47 +/- 3.18 ppm), while alkasite (22.91 +/- 2.64 ppm) and giomer (18.35 +/- 2.21 ppm) released significantly lower fluoride ($p < 0.001$) (Table 3). Pearson correlation showed a moderate positive association between cumulative fluoride release and bacterial reduction ($r = 0.64$, $p < 0.001$), suggesting that greater ion release was associated with better antibacterial performance, although fluoride release alone did not fully explain biofilm inhibition.

Table 3: Fluoride release pattern of tested restorative materials

Material group	Day 1 (ppm)	Day 7 (ppm)	Day 14 (ppm)	Day 28 cumulative (ppm)	p-value
Conventional glass ionomer cement	14.82 +/- 1.46	11.26 +/- 1.08	7.88 +/- 0.82	39.62 +/- 3.74	<0.001
Resin-modified glass ionomer cement	13.10 +/- 1.21	9.84 +/- 0.96	7.02 +/- 0.78	35.47 +/- 3.18	
Giomer restorative material	6.76 +/- 0.74	4.93 +/- 0.61	3.41 +/- 0.49	18.35 +/- 2.21	
Alkasite bioactive restorative material	8.42 +/- 0.88	6.31 +/- 0.70	4.56 +/- 0.52	22.91 +/- 2.64	

DISCUSSION

The present study compared antibacterial efficacy and fluoride release of four pediatric restorative materials under standardized laboratory conditions. The findings rejected the null hypothesis because significant intergroup differences were observed for inhibition zone, direct-contact bacterial reduction, biofilm biomass, and fluoride release. Glass-ionomer-based materials, particularly RMGIC and CGIC, showed superior antibacterial behavior and fluoride release compared with giomer and alkasite restorative materials. These results are consistent with earlier evidence that glass-ionomer materials exhibit greater immediate fluoride availability and measurable inhibition of S. mutans in laboratory models [11].

The greater inhibition zones produced by RMGIC and CGIC may be attributed to early fluoride release, low initial pH during setting, and diffusion of soluble ionic components into the agar medium. Hotwani et al. reported that hybrid tooth-colored restorative materials differ in their antibacterial effects against S. mutans, with glass-ionomer-containing materials generally showing greater inhibition than resin-based materials [12]. Kumar et al. similarly demonstrated antibacterial properties of fluoride-releasing glass-ionomer cements against S. mutans, supporting the clinical rationale for their use in caries-prone children [13]. In the present study, RMGIC demonstrated slightly greater antibacterial activity than CGIC, possibly because of a combined effect of fluoride release, resin-modified matrix characteristics, and better early surface integrity.

The antibacterial performance of the alkasite restorative material was moderate. Its inhibition zone and direct-contact reduction were lower than those of CGIC and RMGIC but higher than those of giomer. This finding agrees with the concept that bioactive or alkasite materials may release alkaline ions and fluoride, but

their antimicrobial effect depends on filler composition, matrix permeability, water sorption, and environmental acidity. Conti et al. reported that bioactive restorative materials may show antibacterial activity against S. mutans, but their performance varies according to testing method and exposure time [14]. The moderate effect observed in the present study suggests that alkasite may be useful where improved handling and esthetics are desired, but it should not be assumed to provide the same fluoride-mediated antibacterial effect as glass-ionomer materials.

Giomer showed the lowest antibacterial performance and fluoride release among the tested materials. This does not necessarily indicate poor clinical performance, because giomers are valued for esthetics, polishability, and potential fluoride recharge. However, their fluoride release is often lower than that of conventional or resin-modified glass ionomer cements. Feiz et al. compared tooth-colored restorative materials and observed significant differences in both antibacterial activity and fluoride release among RMGIC, zirconomer, giomer, and Cention N [15]. The relatively lower giomer performance in the present study may reflect the lower availability of free fluoride ions from pre-reacted glass fillers during the initial observation period.

Biofilm biomass results are clinically important because recurrent caries is more closely related to persistent biofilm at restoration margins than to planktonic bacteria alone. In the present study, lower crystal violet optical density values were observed for RMGIC and CGIC, suggesting reduced adherent biomass. However, the correlation between fluoride release and bacterial reduction was moderate rather than strong. This indicates that antibacterial performance is multifactorial. Surface roughness, surface free energy, hydrophilicity, filler exposure, pH change, and matrix degradation may influence bacterial adhesion independent of fluoride release. Daabash et al. emphasized that surface properties significantly affect S. mutans adhesion to ion-releasing resin-based composites [16]. Therefore, a material with lower fluoride release may still perform acceptably if its surface discourages bacterial attachment, while a high-fluoride material with a rough surface may accumulate plaque clinically.

The fluoride-release pattern observed in this study showed an initial burst followed by gradual reduction, which is typical for ion-releasing restorative materials. CGIC produced the highest cumulative fluoride release, followed closely by RMGIC. This pattern can be explained by rapid surface washout of fluoride ions during early maturation and slower diffusion from the bulk material over time. Kelić et al. showed that resinous coatings can influence ion release from fluoride-releasing restorative materials, indicating that clinical finishing, glazing, and coating procedures may modify the preventive potential of restorations [17]. In pediatric dentistry, this is particularly relevant because surface protection is often recommended for glass ionomer restorations during early setting, but excessive coating thickness may reduce immediate fluoride availability.

Recent pediatric material studies also support the need for careful interpretation of fluoride-release values. Aliberti et al. demonstrated that pediatric restorative materials differ not only in total fluoride release but also in release kinetics across storage periods [9]. Similarly, recent systematic review evidence suggests that bioactive resin composites may show antimicrobial potential in vitro, but available studies remain heterogeneous in design, microbial strain, specimen aging, and outcome measurement [18]. The present study attempted to reduce heterogeneity by testing all materials under the same specimen dimensions, incubation conditions, and measurement intervals.

Clinically, the findings suggest that RMGIC and CGIC may be preferable in high-caries-risk pediatric patients, deep occlusal lesions in primary molars, cervical restorations, and situations where fluoride release and antibacterial effect are priorities. RMGIC may offer an advantageous balance of fluoride release, early strength, and handling. CGIC remains valuable in atraumatic and minimally invasive restorative approaches, especially where moisture control is limited. Alkasite restorative material may be considered when moderate ion release and improved esthetics are desired. Giomer may be suitable for esthetic situations but may require fluoride recharge strategies in high-risk children. [19-22]

This study has limitations. Only one strain of S. mutans was used, whereas dental caries involves complex multispecies biofilms. The oral environment includes salivary proteins, pH fluctuations, dietary sugars, toothbrushing abrasion, fluoride exposure, and thermal changes that cannot be fully reproduced in vitro. The study also evaluated fluoride release in deionized water, which may not represent release in artificial saliva or dynamic pH-cycling conditions. Mechanical properties, marginal sealing, wear resistance, and

long-term fluoride recharge were not assessed. Future studies should include multispecies biofilm models, pH cycling, fluoride recharge, surface roughness analysis, aging protocols, and clinical trials in high-caries-risk pediatric populations.

CONCLUSION

Within the limitations of this in vitro study, resin-modified glass ionomer cement and conventional glass ionomer cement demonstrated the highest antibacterial efficacy against Streptococcus mutans and the greatest cumulative fluoride release over 28 days. Alkasite bioactive restorative material showed moderate antibacterial activity and fluoride release, while giomer showed comparatively lower values during the tested period. The results suggest that glass-ionomer-based materials remain advantageous for high-caries-risk pediatric patients where ion release and antibacterial action are major clinical priorities. Material selection should be individualized according to caries risk, cavity location, moisture control, esthetic demand, and expected longevity.

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