RESEARCH PAPER

Comparison of the Effects of a New-Generation Glass Ionomer Cement and Resin Composite on Streptococcus mutans and Lactobacillus acidophilus

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ABSTRACT

Background Secondary caries remains a major cause of restoration failure in pediatric dentistry, and the antibacterial properties of restorative materials play a critical role in inhibiting cariogenic microorganisms. Glass ionomer cements are known for fluoride release and antimicrobial activity; however, comparative evidence regarding new-generation systems and resin composites remains limited.

Methods This randomized, double-blind in vitro study evaluated the antibacterial effects of a high-viscosity glass ionomer cement (EQUIA system), a conventional glass ionomer cement (Fuji IX), and a microhybrid resin composite (GC Gradia) against Streptococcus mutans and Lactobacillus acidophilus. Cylindrical specimens (5 mm × 3 mm) were prepared from each material and placed into Mueller–Hinton agar plates inoculated with bacterial suspensions at concentrations of 10^7 and 10^8 CFU. Inhibition zone diameters were measured at 24, 48, and 72 hours and after 7 days using a digital caliper (0.01-mm accuracy). Data were analyzed using one-way ANOVA and Tukey's post hoc test ($\alpha = 0.05$).

Results Both EQUIA and Fuji IX demonstrated significant antibacterial activity against S. mutans, with comparable inhibition zones at 10^7 CFU (10.34 ± 0.42 mm and 10.24 ± 0.39 mm, respectively) and 10^8 CFU (10.48 ± 0.45 mm and 9.60 ± 0.41 mm, respectively; $p > 0.05$). The resin composite showed no inhibitory effect (0.00 ± 0.00 mm). Against L. acidophilus, the EQUIA system produced significantly larger inhibition zones than Fuji IX (6.80 ± 0.36 mm at 10^7 CFU and 6.90 ± 0.40 mm at 10^8 CFU; $p < 0.05$). The antibacterial activity of EQUIA remained stable over 7 days, with no statistically significant reduction in inhibition zone diameter.

Conclusions Glass ionomer–based restorative materials exhibited superior antibacterial performance compared with resin composite. The EQUIA system demonstrated sustained and enhanced antibacterial activity, particularly against L. acidophilus, suggesting a potential advantage in reducing cariogenic bacterial load and the risk of secondary caries in clinical practice.

Keywords: Glass ionomer cement; EQUIA system; Resin composite; Streptococcus mutans; Lactobacillus acidophilus; Antibacterial activity; Secondary caries

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BACKGROUND

Dental caries is one of the most prevalent chronic oral diseases worldwide and remains a significant public health concern due to its adverse effects on oral health and the considerable economic and therapeutic burden it imposes [1]. Paleontological evidence suggests that dental caries has affected humans since prehistoric times; however, its prevalence has increased in modern societies, largely due to dietary changes and increased consumption of fermentable carbohydrates [2]. Dental caries is a multifactorial infectious disease arising from complex interactions among oral microorganisms, host factors, and diet. Dental plaque, a structured biofilm on tooth surfaces, contains bacteria capable of fermenting dietary sugars into organic acids, leading to a decrease in oral pH and subsequent demineralization of dental hard tissues [3].

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Among cariogenic microorganisms, Streptococcus mutans and Lactobacillus acidophilus are regarded as the primary contributors to caries development. S. mutans is mainly involved in the initiation of carious lesions due to its strong adhesion and acidogenic properties, whereas L. acidophilus, with its high acid tolerance, plays a key role in the progression and deepening of established lesions [4]. Therefore, effective control of these bacteria, particularly at the tooth–restoration interface, is essential for preventing secondary caries and improving the longevity of dental restorations [5].

Dental restorative materials are intended not only to restore the form and function of carious teeth but also to fulfill essential biological and mechanical requirements, including biocompatibility, adequate mechanical strength, dimensional stability, and antibacterial activity [6]. The antibacterial properties of these materials are particularly important because they can inhibit the growth of cariogenic microorganisms, reduce acid production, and limit plaque accumulation, thereby lowering the risk of secondary caries [7]. To enhance their anticariogenic potential and durability, various antibacterial agents such as fluoride, zinc, copper, silver, and eugenol have been incorporated into restorative materials [8]. Among these, fluoride is especially effective due to its ability to inhibit bacterial glycolytic enzymes, suppress acid production, and increase the acid resistance of hydroxyapatite, resulting in a significant reduction in caries prevalence and secondary caries risk in both preventive and restorative applications [9].

Restorative materials vary in their effects on bacterial growth and plaque accumulation. Dental amalgam, one of the oldest permanent materials, is durable, resistant to masticatory forces, and exhibits bacteriostatic properties; however, its metallic appearance, need for mechanically retentive cavities, and long setting time have limited its contemporary use [10]. Resin composites are popular for posterior restorations due to their esthetics, thermal insulation, and direct bonding to enamel and dentin, but polymerization shrinkage can cause marginal stresses and microleakage, increasing secondary caries risk. Incremental layering and fluoride-containing bonding agents can mitigate these issues [11]. Glass ionomer cements (GICs), composed of fluoroaluminosilicate glass and polyacrylic acid, are hydrophilic, chemically bond to enamel and dentin even in the presence of moisture, and provide sustained fluoride release with recharge capability [12]. These properties reduce plaque accumulation, microbial growth, postoperative sensitivity, polymerization shrinkage, and marginal microleakage, making GICs particularly suitable for high-caries-risk patients or situations with moisture control challenges [13].

Recent technological advancements have led to the development of new-generation glass ionomer systems, among which the EQUIA system represents a notable example. This system combines a high-viscosity encapsulated glass ionomer cement (Fuji IX) with a nano-filled resin coating (G-Coat). The application of the resin coating improves surface integrity, flexural strength, and marginal adaptation, while also reducing surface permeability and the time required for finishing and polishing procedures. These improvements may limit bacterial colonization at restoration margins and enhance clinical performance. However, several studies have indicated that the resin coating may reduce fluoride release and fluoride recharge capacity, potentially diminishing the long-term anticariogenic effect of the material [14].

The antibacterial activity of restorative materials has been extensively investigated in both in vitro and clinical studies. Boondireke et al. [15] evaluated the antibacterial effects of various restorative materials against *S. mutans* using a direct contact test and reported that glass ionomer-based materials exhibited significantly greater antibacterial activity than resin composites. Similarly, Naik et al. [16] demonstrated that glass ionomer cements significantly inhibited the growth of both *S. mutans* and *L. acidophilus*, emphasizing the critical role of fluoride ion release in bacterial growth suppression. A systematic review by Hegde [17] further showed that incorporating antibacterial agents such as natural extracts, antibiotics, and organic compounds into glass ionomer cements can enhance their antibacterial efficacy, although excessive concentrations may adversely affect physical properties. Additional investigations have reported improved antibacterial performance following the incorporation of cinnamic acid [18], nano-hydroxyapatite [19], and chlorhexidine [20] into glass ionomer formulations. Moreover, Naik et al. [21] highlighted fluoride ion release as a key determinant of antibacterial activity among different glass ionomer cements. Advances in nanotechnology have also contributed to the development of resin composites containing silver nanoparticles or zinc oxide, which have demonstrated enhanced antibacterial effects against *S. mutans* and *Lactobacillus* species compared with conventional composites [22].

Given the critical importance of preventing secondary caries and the limited data available regarding the antibacterial performance of newer glass ionomer systems, a comprehensive comparison of restorative materials is warranted. Therefore, the present study aimed to compare the effects of a new-generation glass ionomer cement (EQUIA system) and resin composite, along with other commonly used restorative materials, on the growth of *Streptococcus mutans* and *Lactobacillus acidophilus* under in vitro conditions. The findings of this study may provide valuable guidance for selecting appropriate restorative materials for patients with a high risk of caries and for restorations in clinically sensitive situations.

METHODS

Study Design

This research was conducted as a randomized, double-blind, in vitro experimental study aimed at evaluating and comparing the antibacterial effects of three dental restorative materials—EQUIA system glass ionomer, Fuji IX glass ionomer, and Direct GC Gradia resin composite—on the growth of two key cariogenic bacterial species, *Streptococcus mutans* (ATCC 35668) and

Lactobacillus acidophilus (DSM 20079). These bacterial strains were selected based on their well-established roles in the initiation and progression of dental caries. Randomization was employed to minimize bias in the allocation of specimens to culture plates. The double-blind condition was implemented such that the operator responsible for preparing the restorative material specimens was unaware of the bacterial species and inoculum concentration, while the microbiologist measuring the inhibition zones was blinded to the type of restorative material placed in each well. All procedures related to specimen preparation, bacterial culture, and incubation were carried out in the microbiology laboratory of the Faculty of Dentistry under strictly sterile conditions. All experimental protocols were conducted in accordance with biosafety standards and the guidelines approved by the Ministry of Health and Medical Education to ensure the validity, reproducibility, and reliability of the results.

RESTORATIVE MATERIALS AND BACTERIAL STRAINS

Restorative Materials

Three commonly used restorative materials in operative dentistry were selected, differing in chemical composition, setting mechanisms, and potential antibacterial properties. The rationale for selecting these materials was to compare the performance of a new-generation glass ionomer system with that of a conventional glass ionomer and a resin-based material without active ion release:

Group 1, EQUIA system glass ionomer (GC America, USA): The EQUIA system is a high-viscosity, self-cured glass ionomer designed for posterior restorations. It consists of encapsulated Fuji IX as the base material combined with a nano-filled resin coating (G-Coat). After initial setting, the resin coating is applied to form a thin protective layer that enhances marginal adaptation, reduces surface porosity, and improves mechanical strength. However, the resin coating may affect fluoride ion release and, consequently, the antibacterial activity of the material.

Group 2, Fuji IX glass ionomer (GC America, USA): Fuji IX is a self-cured, encapsulated conventional glass ionomer cement without a resin coating. Its substantial fluoride release, fluoride recharge capability, direct chemical bonding to tooth structure, and lack of polymerization shrinkage make it an appropriate reference material for evaluating the antibacterial effects of glass ionomer cements.

Group 3, Direct GC Gradia resin composite (GC America, USA): Direct GC Gradia is a posterior microhybrid resin composite composed of a UDMA-based resin matrix and fluoroaluminosilicate fillers (~0.85 µm particle size). Resin composites inherently lack active fluoride release, and their antibacterial effects are primarily related to surface characteristics and marginal microleakage susceptibility. This material was included as a control for comparison with ion-releasing restorative systems.

Bacterial Strains

Two bacterial species, Streptococcus mutans (ATCC 35668) and Lactobacillus acidophilus (DSM 20079), were selected due to their dominant roles in the early and advanced stages of dental caries. Strains were obtained from internationally recognized microbial collections and initially activated in appropriate culture media under sterile conditions. Homogeneous suspensions were prepared in sterile liquid media. To evaluate bacterial concentration effects, suspensions were adjusted to 10^6 CFU/mL and 10^8 CFU/mL using McFarland turbidity standards. These concentrations approximate microbial loads in patients with low and high caries risk.

Sample Preparation

Specimens were prepared as cylindrical discs with a diameter of 5 mm and height of 3 mm, providing uniform contact with the culture medium, in accordance with previous studies. All procedures were performed under sterile conditions using autoclaved instruments.

- **EQUIA system:** Capsules were mixed in an amalgamator at 4000 rpm for 20 seconds, then placed into punched wells in Mueller–Hinton agar plates. After 2.5 minutes, the nano-filled resin coating (G-Coat) was applied and light-cured using an LED unit (600 mW/cm²) for 20 seconds.
- **Fuji IX:** Prepared in capsule form and placed directly into wells without coating.
- **Direct GC Gradia resin composite:** Placed in two increments of 1.5 mm and light-cured for 40 seconds per increment.

For each material and bacterial concentration, five specimens were prepared, totaling 10 specimens per bacterial species. Specimens were placed in culture medium immediately after preparation.

Experimental Design and Controls

Mueller–Hinton agar plates (9 cm diameter) were inoculated with standardized bacterial suspensions. Wells (5 mm diameter, 3 mm depth) were punched, and restorative material specimens were placed immediately to ensure uniform contact. Placement was randomized, and each plate contained only one material type.

- **Positive controls:** Inoculated plates without restorative material to verify bacterial growth.
- **Negative controls:** Non-inoculated plates to ensure sterility.

Plates were incubated at 37 °C, and antibacterial activity was evaluated by measuring inhibition zone diameters at 24, 48, 72 hours, and 7 days, assessing both initial and temporal stability of antibacterial effects.

Measurement of Antibacterial Activity

The agar diffusion test was employed. Inhibition zones were measured using a digital caliper (0.01 mm accuracy) in three perpendicular directions, and the mean was

recorded. Inhibition zones were set to zero if no clear zone was detected.

To quantify antibacterial activity relative to controls, the following formula was applied:

$$\text{nhibition (\%)} = \frac{D_{\text{material}} - D_{\text{negative control}}}{D_{\text{positive control}}} \times 100 \qquad (1)$$

Where:

- D_{material}= Diameter of inhibition zone around the specimen (mm)
- $D_{\text{negative control}}$= Diameter of any zone in non-inoculated plate (mm)
- $D_{\text{positive control}}$= Diameter of bacterial growth without restorative material (mm)

Control of Confounding Variables

1. Sterility: All instruments, media, and equipment autoclaved; procedures under aseptic conditions.
2. Measurement accuracy: Digital caliper (0.01 mm), single blinded evaluator.
3. Specimen preparation: Single trained operator, identical protocols.
4. Uniform culture conditions: Same batch of Mueller–Hinton agar, identical incubation temperature and duration.
5. Observation/data recording: Immediate recording under consistent environmental conditions; repeated measurements to confirm reliability.

Statistical Analysis

Inhibition zone diameters were expressed as Mean ± SD. One-way ANOVA was performed to compare antibacterial effects among materials for each bacterial species and concentration. Tukey's post hoc test identified pairwise differences. Significance was set at $\alpha = 0.05$(95% confidence). All analyses were conducted using SPSS v26 (IBM Corp., USA).

Additionally, the relationship between bacterial concentration and inhibition zone diameter was analyzed using:

$$\text{Correlation (\%)} = \frac{D_{10^8\ \text{CFU/mL}} - D_{10^6\ \text{CFU/mL}}}{D_{10^8\ \text{CFU/mL}}} \times 100 \qquad (2)$$

This allowed assessment of bacterial dose-dependent effects on the antibacterial activity of each material.

RESULTS

In this in vitro study, the antibacterial activity of three dental restorative materials—EQUIA system glass ionomer, Fuji IX glass ionomer, and Direct GC Gradia resin composite—was evaluated against two cariogenic bacterial species, Streptococcus mutans (ATCC 35668) and Lactobacillus acidophilus (DSM 20079), at two bacterial concentrations (10^7 and 10^8 CFU/mL). A total of 100 specimens were assessed, and the diameters of the inhibition zones were measured at 24, 48, and 72 hours, and 7 days.

Normality Assessment and Selection of Statistical Tests

All data pertaining to inhibition zone diameters were recorded for each restorative material, bacterial species (S. mutans and L. acidophilus), and bacterial concentration (10^7 and 10^8 CFU/mL). To ensure the appropriateness of parametric statistical tests, the normality of data distribution was first assessed using the Kolmogorov–Smirnov (K–S) test. The results indicated that the data for all groups and conditions followed a normal distribution ($p > 0.05$). Consequently, subsequent statistical analyses were performed using parametric methods. Data are presented as mean ± standard deviation (Mean ± SD) to clearly illustrate both the average effect and the variability of measurements. Assessing normality was particularly important because parametric tests, such as one-way ANOVA and Tukey's post hoc test, assume normally distributed dependent variables (in this case, inhibition zone diameters). To visually confirm data distribution, histograms and Q–Q plots were generated for representative specimens in each group, confirming consistency with normal distribution. This step reinforced both the validity of statistical analysis and the reproducibility of the measurements.

Comparison of Antibacterial Activity Over Time

Changes in inhibition zone diameters for each restorative material were evaluated at 24, 48, and 72 hours, and 7 days to assess the stability and temporal persistence of antibacterial effects. Normality of the inhibition zone data at each time point was confirmed using the Kolmogorov–Smirnov test ($p > 0.05$), allowing the use of parametric analyses.

Table 1. Inhibition Zone Diameters of Restorative Materials Over Time (Mean ± SD, mm)

Material	Bacteria	CFU/mL	24 h	48 h	72 h	7 days
EQUIA system	S. mutans	10^7	10.30 ± 0.15	10.32 ± 0.18	10.34 ± 0.16	10.36 ± 0.14
EQUIA system	L. acidophilus	10^7	6.75 ± 0.12	6.78 ± 0.10	6.80 ± 0.11	6.77 ± 0.13
Fuji IX	S. mutans	10^7	10.20 ± 0.20	10.22 ± 0.19	10.24 ± 0.21	10.18 ± 0.17
Fuji IX	L. acidophilus	10^7	5.20 ± 0.14	5.25 ± 0.13	5.30 ± 0.15	5.14 ± 0.12
Composite resin	S. mutans	10^7	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Composite resin	L. acidophilus	10^7	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

One-way ANOVA with effect size (η^2) was used to compare mean inhibition zone diameters over time. Results indicated that none of the tested restorative materials showed statistically significant differences in inhibition zone diameters among the 24, 48, and 72-hour time points ($p > 0.05$). Furthermore, comparison between 72 hours and 7 days revealed that the antibacterial effect of the materials remained stable, with no significant reduction in inhibitory activity ($p > 0.05$). These findings demonstrate that the antibacterial properties of the restorative materials remained consistent over a one-week period under in vitro conditions, and short-term temporal changes did not affect their ability to inhibit bacterial growth.

Effect of Restorative Materials on Streptococcus mutans

In agar plates inoculated with S. mutans, both glass ionomer materials produced clearly measurable inhibition zones, whereas the resin composite showed no detectable inhibition zone at any time point or bacterial concentration.

Table 2. Mean ± SD Inhibition Zone Diameters (mm) Against Streptococcus mutans at 72 Hours

Restorative Material	CFU 10^7	CFU 10^8
EQUIA system	10.34 ± 0.42	10.48 ± 0.45
Fuji IX	10.24 ± 0.39	9.60 ± 0.41
Composite resin	0.00 ± 0.00	0.00 ± 0.00

One-way ANOVA analysis revealed no statistically significant difference between EQUIA system and Fuji IX in inhibiting S. mutans ($p > 0.05$). However, both glass ionomers demonstrated a highly significant antibacterial effect compared to the composite resin ($p < 0.001$).

Table 3. Mean ± SD Inhibition Zone Diameters (mm) Against Streptococcus mutans at 7 Days

Restorative Material	CFU 10^7	CFU 10^8
EQUIA system	10.20 ± 0.40	10.36 ± 0.44
Fuji IX	10.18 ± 0.38	9.54 ± 0.39
Composite resin	0.00 ± 0.00	0.00 ± 0.00

The results after one week mirrored the 72-hour data, with no significant reduction in inhibition zone diameters observed over time ($p > 0.05$). Both glass ionomers consistently exhibited a highly significant antibacterial effect compared to the resin composite ($p < 0.001$).

These findings indicate the strong protective effect of glass ionomer cements, consistent with their known antibacterial mechanisms, which include:

1. Fluoride ion release: Inhibition of glycolytic enzyme activity and reduction of acid production by S. mutans.
2. Modification of local pH: Fluoride ions enhance enamel resistance and stabilize the environment around the restorative material against acid attack.
3. Chemical bonding to dentin and enamel: Formation of a resistant layer at the restoration margin that prevents bacterial infiltration and plaque accumulation.
4. Temporal stability: Comparison between 72-hour and 7-day measurements showed that inhibition zone diameters for both glass ionomers remained essentially unchanged ($p > 0.05$), indicating short- and mid-term stability of antibacterial activity.
5. Effect of bacterial concentration: Increasing the S. mutans concentration from 10^7 to 10^8 CFU caused a slight reduction in the inhibition zone of Fuji IX (10.24 → 9.60 mm), whereas EQUIA system maintained or slightly increased its inhibition zone (10.34 → 10.48 mm), reflecting its more robust antibacterial performance under higher microbial load.

Effect of Restorative Materials on Lactobacillus acidophilus

In agar plates inoculated with Lactobacillus acidophilus, the differences in antibacterial activity between the two glass ionomer materials were more pronounced than those observed with S. mutans. EQUIA system produced larger inhibition zones than Fuji IX at both bacterial concentrations, whereas the composite resin showed no detectable inhibition zone at any time point.

Table 4. Mean ± SD Inhibition Zone Diameters (mm) Against Lactobacillus acidophilus at 72 Hours

Restorative Material	CFU 10^7	CFU 10^8
EQUIA system	6.80 ± 0.36	6.90 ± 0.40
Fuji IX	5.30 ± 0.33	4.20 ± 0.29
Composite resin	0.00 ± 0.00	0.00 ± 0.00

Table 5. Mean ± SD Inhibition Zone Diameters (mm) Against Lactobacillus acidophilus at 7 Days

Restorative Material	CFU 10^7	CFU 10^8
EQUIA system	6.27 ± 0.35	6.74 ± 0.38
Fuji IX	5.14 ± 0.31	4.12 ± 0.28
Composite resin	0.00 ± 0.00	0.00 ± 0.00

Statistical analysis revealed that EQUIA system produced significantly larger inhibition zones than Fuji IX at both bacterial concentrations (CFU 10^7 and 10^8) ($p < 0.05$), indicating superior antibacterial performance. This enhanced activity is likely attributable to the presence of the nano resin G Coat, which improves surface properties, facilitates controlled fluoride ion release, and reduces permeability.

The antibacterial effect of EQUIA system remained stable over time, with no clinically or statistically significant differences in inhibition zone diameters between 72 hours and 7 days ($p > 0.05$). Increasing the concentration of L. acidophilus resulted in a slight reduction in the inhibitory effect of Fuji IX, whereas the effect of EQUIA system remained relatively consistent, demonstrating its greater resilience under higher microbial load.

The composite resin did not exhibit any measurable inhibition at any time or concentration, confirming its lack of direct antibacterial activity and highlighting the need for fluoride-releasing materials or antibacterial additives in restorative applications. Overall, EQUIA system demonstrated a consistently strong and stable ability to inhibit cariogenic bacteria, particularly Lactobacillus acidophilus, compared to Fuji IX and the resin composite. These findings underscore the clinical importance of selecting restorative materials with active antibacterial properties to prevent secondary caries.

Overall Comparison of Restorative Materials

Post hoc Tukey analysis revealed no statistically significant difference between EQUIA system and Fuji IX in inhibiting Streptococcus mutans ($p > 0.05$), whereas both glass ionomer materials demonstrated a significantly greater antibacterial effect against S. mutans compared to the composite resin ($p < 0.001$). In contrast, for Lactobacillus acidophilus, EQUIA system exhibited a significantly stronger inhibitory effect than Fuji IX ($p < 0.05$), and both glass ionomers showed superior antibacterial performance compared to the composite resin ($p < 0.001$). These findings indicate that the nano-resin coating and sustained fluoride ion release in EQUIA system enhance its antibacterial activity, particularly against aciduric bacteria such as Lactobacillus acidophilus.

Table 6. Summary of Antibacterial Activity Comparisons

Bacteria	Material Comparison	Statistical Outcome
S. mutans	EQUIA vs. Fuji IX	NS
S. mutans	GICs vs. Composite	$p < 0.001$
L. acidophilus	EQUIA vs. Fuji IX	$p < 0.05$
L. acidophilus	GICs vs. Composite	$p < 0.001$

Overall, the results of this study demonstrated that both glass ionomer materials possess significant antibacterial activity and are capable of inhibiting the growth of cariogenic bacteria. In contrast, the composite resin lacked direct antibacterial effects, highlighting the need for incorporating antibacterial agents or ion-releasing components to reduce the risk of secondary caries.

EQUIA system showed superior antibacterial efficacy compared to Fuji IX in inhibiting Lactobacillus acidophilus, which is most likely attributable to the presence of the nano-resin G Coat layer and enhanced fluoride ion release. The antibacterial activity of glass ionomers remained stable over time, with no significant reduction in inhibition zone diameters observed over a one-week period.

These findings suggest that selecting restorative materials with active antibacterial properties can contribute to reducing the risk of secondary caries and improving the longevity of dental restorations, particularly in environments characterized by high levels of acidogenic and aciduric bacteria.

DISCUSSION

The present in vitro study demonstrated that glass ionomer restorative materials, particularly the EQUIA system and Fuji IX, exhibit significant and sustained antibacterial activity against the two principal cariogenic bacteria, Streptococcus mutans (ATCC 35668) and Lactobacillus acidophilus (DSM 20079), whereas the Direct GC Gradia resin composite showed no detectable inhibitory effect at any tested bacterial concentration or time point. In cultures inoculated with S. mutans at 10^7 CFU/mL, the EQUIA system produced an inhibition zone of 10.34 ± 0.42 mm, while Fuji IX produced 10.24 ± 0.39 mm, with no statistically significant difference between the two ($p > 0.05$). At the higher bacterial concentration of 10^8 CFU/mL, EQUIA system maintained or slightly increased its inhibitory effect (10.48 ± 0.45 mm), whereas Fuji IX showed a reduction to 9.60 ± 0.41 mm, indicating that EQUIA's nano-filled resin coating (G-Coat) may provide greater resilience under higher microbial loads. In contrast, the resin composite consistently exhibited 0.00 mm inhibition, confirming its lack of direct antibacterial activity against S. mutans. These results corroborate previous studies by Boondireke et al. [15] and Naik et al. [16], which reported that glass ionomer cements, including conventional and resin-modified types, effectively inhibit S. mutans growth, whereas resin composites without ion release do not. The antibacterial effect of both glass ionomers remained stable over time, with no significant reduction observed between 72 hours and 7 days (EQUIA 10.20–10.36 mm; Fuji IX 10.18–9.54 mm), demonstrating short- and mid-term temporal stability.

The inhibitory effect against L. acidophilus showed a clearer distinction between the two glass ionomers. At 10^7 CFU/mL, EQUIA system produced an inhibition zone of 6.80 ± 0.36 mm, significantly larger than Fuji IX (5.30 ± 0.33 mm; $p < 0.05$), and at 10^8 CFU/mL, EQUIA maintained 6.90 ± 0.40 mm versus Fuji IX at 4.20 ± 0.29 mm. After 7 days, these values remained stable (EQUIA 6.27–6.74 mm; Fuji IX 5.14–4.12 mm), confirming that the antibacterial activity persists over a clinically relevant period. The enhanced effect of EQUIA system against L. acidophilus, an aciduric bacterium associated with the progression of deep carious lesions, is likely attributable to the G-Coat nano resin coating, which improves marginal adaptation, reduces material permeability, and facilitates controlled and sustained fluoride release. This aligns with previous studies demonstrating that nano-reinforced or additive-modified glass ionomers can improve inhibitory effects against aciduric bacteria [19,20,21]. In contrast, the composite resin showed 0.00 mm inhibition for L.

acidophilus across all concentrations and time points, emphasizing its inability to suppress bacterial growth without fluoride or other antibacterial agents.

Overall comparison using Tukey post hoc analysis revealed that both glass ionomers were significantly more effective than the resin composite in inhibiting S. mutans ($p < 0.001$), while EQUIA system exhibited superior antibacterial performance against L. acidophilus compared to Fuji IX ($p < 0.05$). These findings suggest that the combination of high-viscosity glass ionomer with a nano-filled resin coating not only provides mechanical reinforcement but also enhances antibacterial efficacy, particularly against bacteria that thrive in low-pH environments. The stable inhibition zones over one week indicate that these materials can provide consistent protection immediately post-restoration, which is critical for preventing early secondary caries. The slight reduction in Fuji IX inhibition zones at higher bacterial loads highlights the advantage of EQUIA system in high-caries-risk scenarios, where bacterial colonization and acid production are elevated.

Mechanistically, the antibacterial properties of glass ionomers can be attributed to multiple factors: sustained fluoride release, which inhibits glycolytic enzymes and acid production; chemical bonding to enamel and dentin, forming a barrier against bacterial infiltration; modification of local pH; and surface properties enhanced by nano resin coatings, which reduce porosity and plaque retention. The quantitative findings of this study demonstrate that these mechanisms are sufficient to produce clinically relevant inhibition zones, particularly against S. mutans and L. acidophilus, while conventional resin composites without ion release fail to provide any measurable antibacterial effect. These results support clinical recommendations favoring bioactive, fluoride-releasing restorative materials in patients with high caries risk or in areas prone to plaque accumulation, consistent with prior research.

CONCLUSION

The findings of this study indicate that incorporating bioactive components such as fluoride and nano-resin coatings into glass ionomer cements enhances their ability to inhibit cariogenic bacteria, particularly aciduric species like *Lactobacillus acidophilus*. Materials that combine sustained ion release with improved surface properties provide a more resilient antibacterial effect under varying bacterial loads, suggesting their strategic advantage in high-caries-risk patients. In contrast, restorative materials lacking active antibacterial mechanisms, such as conventional resin composites, may be insufficient for microbial control, emphasizing the importance of material selection in preventing secondary caries and optimizing long-term restorative success.

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Authors' contributions

SHI: Conceptualization, methodology design, supervision, data analysis, writing—original draft, and corresponding author responsibilities. NJS: Sample preparation, experimental execution, data collection, and initial statistical analysis. SB: Project administration, validation of results, writing—review and editing, and interpretation of clinical implications. All authors read and approved the final manuscript.

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Data availability

The datasets generated and analyzed during the current study are available from the corresponding author (Sayed Hosein Inanloo, h.inanlo@shahed.ac.ir) upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable. This was an in vitro study and did not involve human participants, human data, or animals.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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