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ORIGINAL RESEARCH

Comparative evaluation of fluoride release from glass carbomer and bioactive restorative materials used in pediatric dentistry: an *in vitro* study

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Abstract

Background: This *in vitro* study aimed to comparatively evaluate the fluoride release profiles of glass carbomer and a bioactive restorative material (ACTIVA™ Kids Bioactive Restorative) in comparison with conventional and resin-modified glass ionomer cements used in pediatric dentistry. **Methods**: Conventional glass ionomer cement (Fuji IX), resin-modified glass ionomer cement (Fuji II LC), glass carbomer (GCP Glass Fill), and a bioactive restorative material (ACTIVA™ Kids Bioactive Restorative) were evaluated. Disc-shaped specimens (n = 13 per group; 10 mm diameter, 1.0 mm thickness) were prepared and individually immersed in 10 mL of deionized water at 37 °C. Fluoride release was measured daily during the first 7 days and subsequently on days 14, 21, and 28 using a fluoride ion-selective electrode. Data were analyzed using non-parametric statistical tests (Kruskal-Wallis and Mann-Whitney U tests), with statistical significance set at $p < 0.05$. **Results**: Glass carbomer exhibited the highest fluoride release among all tested materials throughout the experimental period, followed by conventional glass ionomer cement, resin-modified glass ionomer cement, and ACTIVA™ Kids Bioactive Restorative. The highest fluoride release for all materials was observed on day 1, followed by a gradual decrease over time. While no significant differences were detected among materials on day 1 ($p > 0.05$), statistically significant differences were observed at subsequent time points ($p < 0.05$). **Conclusions**: Within the limitations of this *in vitro* study, glass carbomer cements demonstrated higher fluoride release compared with the other evaluated restorative materials. These findings may be relevant for restorative material selection in pediatric patients with high caries risk; however, clinical extrapolation should be made with caution.

Keywords

Fluoride ion release; Glass ionomer cements; Glass carbomer; Bioactive restorative material; Pediatric dentistry; Caries prevention

1. Introduction

Dental caries, despite being a largely preventable disease, continues to pose a significant public health problem, particularly in children [1]. In Turkey, dental caries remains a major public health concern in children, with regional studies reporting prevalence rates as high as 60–80% [2]. This trend has been corroborated by a recent meta-analysis, which estimated a pooled prevalence of 75.6% among pediatric populations [3, 4]. Fluoride is an effective, safe, and economical agent in the prevention and control of dental caries [5]. Therefore, many products, such as gels, varnishes, toothpastes, and restorative materials contain fluoride, and restorative materials capable of fluoride release are preferred in the dental treatment of both children and adults.

Conventional and resin-modified glass ionomer cements, nano-featured glass ionomer cements, polyacid-modified composite resins, and fluoride-containing composite resins are restorative materials that release fluoride [6]. Conventional glass ionomer cements, which contain polyalkenoic acid and silicate glass, set through an acid-base reaction, during which fluoride is released [6]. In conventional glass ionomer cements, fluoride release is high within the first 24 hours, but decreases over time [6, 7]. Although the ability of conventional glass ionomer cements to release fluoride is an advantage, they also have several disadvantages, including moisture sensitivity, difficult application, low clinical performance, low abrasion resistance, and poor aesthetics [8]. To overcome these disadvantages, resin-modified glass ionomer cements have been developed [8].

Another fluoride-releasing restorative material is glass carbomer, a glass ionomer-type cement containing carbomized nanoparticles [9]. However, it differs from glass ionomers due to its nanosized powder particles and inclusion of fluorapatite

[9]. The addition of nanoparticles improves the mechanical properties of the material. When compared with conventional and resin-modified glass ionomer cements, glass carbomers exhibit longer working time, faster setting time, improved aesthetic performance, and enhanced translucency [10]. In addition, glass carbomers do not contain resin, monomer, metal or Bisphenol-A [9].

Bioactive restorative materials have been developed to combine the mechanical properties of resin-based composites with the fluoride release and ion exchange characteristics of glass ionomer cements [11, 12]. ACTIVA™ Kids Bioactive Restorative is a dual-cure, resin-based material containing a modified polyacrylic acid matrix and reactive fluoroaluminosilicate glass fillers, enabling an acid–base reaction in addition to resin polymerization [13, 14]. This hybrid setting mechanism allows dynamic ion exchange with the surrounding environment and contributes to the release of fluoride, calcium, and phosphate ions, particularly in response to pH changes [14–17]. The hydrophilic ionic resin matrix and the presence of bioactive glass fillers are considered key factors influencing the fluoride release behavior of ACTIVA materials [16, 18]. Fluoride-releasing restorative materials increase the fluoride levels in saliva, plaque and hard dental tissues, acting as a fluoride reservoir and thereby helping to prevent caries formation. Moreover, high concentrations of fluoride inhibit demineralization by preventing bacterial colonization and acid production [18]. Therefore, both the fluoride ion content in the material and the sustained and effective release of fluoride from the material are critical for demonstrating the caries-preventive effect of the restorative material [18–20].

When studies on fluoride release from restorative materials are reviewed, it is observed that although numerous studies have evaluated the fluoride release of glass ionomer–based restorative materials [21–23], limited data are available regarding the fluoride release behavior of newer restorative materials such as glass carbomer and bioactive resin-based materials [15, 24–28]. In particular, comparative data evaluating glass carbomer and ACTIVA™ Kids Bioactive Restorative within the same experimental framework remain scarce. Therefore, the present *in vitro* study aimed to comparatively evaluate the fluoride release profiles of glass carbomer and ACTIVA™ Kids Bioactive Restorative alongside conventional glass ionomer cement and resin-modified glass ionomer cement under standardized experimental conditions.

The novelty of this study lies in the direct and simultaneous comparison of glass carbomer and ACTIVA™ Kids Bioactive Restorative—two restorative materials increasingly used in pediatric dentistry—together with and conventional resin-modified glass ionomer cements under identical and standardized experimental conditions. To the best of the authors' knowledge, such a comparative evaluation of these materials within a single experimental framework remains limited in the current literature. This approach provides clinically relevant insight into the fluoride release behavior of contemporary restorative materials used in pediatric dentistry.

2. Materials and methods

2.1 Study design and experimental setting

This study was designed as an *in vitro* comparative experimental study evaluating the fluoride release profiles of four restorative materials commonly used in pediatric dentistry. All sample preparation procedures and fluoride measurements were performed under standardized laboratory conditions by a single trained operator to minimize operator-related variability. Prior to the experimental procedures, the operator was calibrated through repeated specimen preparation and polishing trials to ensure procedural consistency. The study was conducted at the Eskişehir Osmangazi University Central Research Laboratory Application and Research Center.

2.2 Materials used

The restorative materials evaluated in this study were:

- Conventional glass ionomer cement (Fuji IX, GC Corporation, Tokyo, Japan).
- Resin-modified glass ionomer cement (Fuji II LC, GC Corporation, Tokyo, Japan).
- Glass carbomer (GCP Glass Fill, GCP Dental, Netherlands).
- Bioactive restorative material (ACTIVA™ Kids Bioactive Restorative, Pulpdent Corporation, USA).

Details regarding material composition, manufacturer, and lot numbers are presented in Table 1.

For the purposes of this study, bioactive restorative material refers to materials capable of releasing ions, such as fluoride, calcium, and phosphate, and interacting chemically with the surrounding tooth structure through ion exchange mechanisms, as described in the current literature.

2.3 Sample preparation

A total of 52 disc-shaped specimens were prepared, with 13 samples per material group (n = 13). The sample size was determined based on previous *in vitro* fluoride release studies evaluating glass ionomer–based and bioactive restorative materials, which commonly employed similar sample sizes to detect comparative fluoride release trends over time [16, 29, 30]. Specimens were fabricated using standardized polyethylene molds with a diameter of 10 mm and a thickness of 1.0 mm, consistent with previously published fluoride-release studies. The molds were positioned on a glass slab covered with a cellulose matrix strip to prevent adhesion. All materials were prepared strictly according to the manufacturers' instructions and inserted into the molds. A second cellulose matrix strip and glass slab were placed on top, and gentle manual pressure was applied to extrude excess material and achieve uniform specimen surfaces.

Polymerization protocols are summarized in Table 2. Light-curable materials were polymerized using a light-emitting diode (LED) light-curing unit (Demi Ultra, Kerr Corporation, Orange, CA, USA) with a light intensity of 1100 mW/cm^2. For glass carbomer specimens, light activation was applied to accelerate the initial setting reaction and facilitate specimen handling, in accordance with the manufacturer's

TABLE 1. Materials used in the study.

Material	Type	Composition	Lot Number	Manufacturer
Fuji IX	Conventional glass ionomer cement	Powder: Fluoroaluminosilicate glass, Liquid: Polyacrylic acid, polybasic carboxylic acid, distilled water	1806271	GC Corporation, Tokyo, Japan
Fuji II LC	Resin-modified glass ionomer cement	Fluoroaluminosilicate glass, 2-hydroxyethyl methacrylate, polybasic carboxylic acid, urethane dimethacrylate, camphorquinone, distilled water	190516A	GC Corporation, Tokyo, Japan
GCP Glass Fill	Glass carbomer	Fluoroaluminosilicate glass, nano fluoro/hydroxyapatite, polyacids	71712907	GCP Dental, Mijlweg 7, Netherlands
ACTIVA™ Kids Bioactive-Restorative	Bioactive restorative material	Modified polyacrylic acid with diurethane and other methacrylate mixture, amorphous silica, sodium fluoride, water	190710	Pulpdent Corporation, Watertown, USA

TABLE 2. Preparation and polymerization times of restorative materials.

Material	Preparation Method	Polymerization Time
Fuji IX	Mixing powder and liquid with a plastic spatula for 25–30 seconds	10 minutes under pressure
Fuji II LC	Mixing the capsule with a mixer for 10 seconds and placing it into the applicator gun	20 seconds light-curing
GCP Glass Fill	Mixing the capsule with a mixer for 10–15 seconds and placing it into the applicator gun	20 seconds light-curing
ACTIVA™ Kids Bioactive-Restorative	Placing the paste-paste tube into its specific applicator gun	20 seconds light-curing

recommendations. Although glass carbomer is primarily an acid–base setting material, light activation was used solely to standardize sample preparation and ensure early surface integrity.

2.4 Finishing and storage conditions

After polymerization, specimens were carefully removed from the molds and polished using silicon carbide abrasive papers of 600-, 800-, and 1000-grit, applied sequentially for 30 seconds per grit under light manual pressure. Polishing procedures were performed by the same operator for all specimens to ensure consistency. Following polishing, specimens were weighed using an analytical balance and stored for 24 hours at 37 °C and 100% relative humidity to allow completion of the setting reaction.

Each specimen was then individually immersed in 10 mL of deionized water in labeled polyethylene tubes. During the first week, the storage solutions were replaced daily. Thereafter, solutions were renewed prior to each scheduled measurement. All specimens were stored at 37 °C throughout the experimental period. The pH of the storage medium was not actively adjusted during the storage period. Deionized water was selected as the storage medium to allow standardized comparison of intrinsic fluoride release among materials, acknowledging that this medium may overestimate fluoride release compared with oral conditions.

2.5 Fluoride ion measurement

Fluoride release was measured daily during the first 7 days and subsequently on days 14, 21, and 28. Fluoride ion concentrations were determined using a fluoride ion-selective electrode (ORION 9609BNWP, Ionplus Sure-Flow Fluoride, Thermo Electron Corp., Beverly, MA, USA) connected to a digital ion analyzer (ORION 720A+, Thermo Scientific™., Beverly, MA, USA).

Prior to each measurement session, the fluoride ion-selective electrode was calibrated using standard fluoride solutions with concentrations ranging from 0.1 to 10 ppm. Calibration measurements were performed sequentially from the lowest to the highest fluoride concentration, and the corresponding electrode potentials (mV) were recorded.

A calibration curve was constructed by plotting the measured electrode potentials (mV) against the logarithm of fluoride concentration (log ppm). Preliminary calibration analysis demonstrated that the concentration range between 0.1 and 10 ppm provided optimal linearity, with a correlation coefficient (R^2) closest to 1.0. Therefore, this concentration range was selected for all subsequent calibration procedures. For each measurement day, fresh calibration solutions within this range were prepared, and the corresponding calibration equation and R^2 value ($\geq$0.99) were obtained and used for fluoride concentration calculations. All measurements were conducted at room temperature (23 ± 1 °C).

For analysis, equal volumes of the sample solution and TISAB II buffer were mixed to stabilize ionic strength and pH. The electrode was immersed in the solution until a stable millivolt (mV) reading was obtained, typically within approximately 2 minutes. Measurements were performed in duplicate, and mean values were converted to fluoride concentrations expressed in parts per million (ppm) using the calibration equation. The electrode was rinsed with distilled water and gently dried between measurements to prevent cross-contamination.

2.6 Statistical analysis

Statistical analysis was performed using SPSS software (version 24.0; SPSS Inc., Chicago, IL, USA). Data distribution was assessed using the Shapiro-Wilk test and demonstrated non-normal distribution. Therefore, non-parametric statistical tests were applied. Intergroup comparisons were conducted using the Kruskal-Wallis test, followed by pairwise comparisons with the Mann-Whitney U test when statistically significant differences were detected. Measurements were performed in duplicate, and mean values were used for analysis. Statistical significance was set at $p < 0.05$.

Given the exploratory nature of this *in vitro* study and the limited sample size, no formal correction for multiple comparisons (*e.g.*, Bonferroni adjustment) was applied, as such corrections may increase the risk of Type II error. Accordingly, the results were interpreted cautiously, with emphasis on overall trends and relative differences among materials, rather than isolated *p*-values.

3. Results

The mean fluoride release values (ppm) and standard deviations for all restorative materials evaluated in this study are presented in Table 3. On day 1, no statistically significant differences were observed among the materials ($p > 0.05$).

From day 2 onward, statistically significant differences in fluoride release were detected among the restorative materials at most time points ($p < 0.05$). Excluding the initial measurement day, GCP Glass Fill consistently demonstrated the highest fluoride release values throughout the 28-day evaluation period. Fuji IX exhibited the second highest fluoride release profile, followed by Fuji II LC, while ACTIVA™ Kids Bioactive Restorative generally showed the lowest fluoride release values.

All tested materials exhibited their highest fluoride release on day 1, followed by a gradual decrease over time. Despite this overall declining trend, fluoride release remained detectable for all materials throughout the entire experimental period.

Temporal comparisons within each material group revealed statistically significant differences in fluoride release across the evaluation period ($p < 0.05$). The daily fluoride release patterns of the restorative materials over time are illustrated in Fig. 1.

4. Discussion

Secondary caries remains a prevalent challenge in pediatric dentistry and is a key determinant of the clinical longevity of restorative materials [31]. Given the low incidence of secondary caries observed in restorations utilizing silicate cements, research has increasingly focused on the development of fluoride-releasing restorative materials [6, 31]. Currently, fluoride-releasing restorative materials mainly include conventional glass ionomer cements, glass ionomer–based hybrid materials, glass carbomers, and bioactive restorative materials [21, 32–35]. A review of the literature on fluoride release demonstrates that the majority of studies have concentrated on glass ionomer-based restorative materials [22, 29, 36, 37], whereas investigations on glass carbomer [25, 26, 34, 38] and ACTIVA™ Kids Bioactive Restorative materials remain limited [16, 24, 27]. In this context, the present study aimed to comparatively evaluate the fluoride release capacities of glass carbomer and ACTIVA™ Kids Bioactive Restorative

TABLE 3. Mean and standard deviation of fluoride release (ppm) of the materials.

Days	Fuji IX	Fuji II LC	GCP Glass Fill	ACTIVA™ Kids Bioactive Restorative
Day 1	$16.34 \pm 2.08^{A,1}$	$15.15 \pm 3.52^{A,1}$	$17.33 \pm 1.22^{A,1}$	$15.13 \pm 3.79^{A,1}$
Day 2	$13.20 \pm 2.41^{A,B,2,3}$	$12.37 \pm 1.08^{B,2}$	$14.54 \pm 2.81^{A,2,3,4}$	$9.59 \pm 0.48^{C,2}$
Day 3	$14.24 \pm 2.06^{B,1,2}$	$11.66 \pm 0.97^{C,2}$	$15.63 \pm 0.73^{A,2}$	$9.21 \pm 0.41^{D,2}$
Day 4	$13.42 \pm 2.03^{B,2}$	$11.37 \pm 0.93^{C,2,3}$	$14.93 \pm 0.89^{A,2,3}$	$9.25 \pm 0.38^{D,2}$
Day 5	$12.42 \pm 1.81^{B,2,3}$	$10.45 \pm 0.62^{C,3,4}$	$13.78 \pm 0.81^{A,3,5}$	$8.87 \pm 0.42^{D,2,3}$
Day 6	$11.84 \pm 1.69^{B,3,4}$	$10.24 \pm 0.53^{C,4}$	$13.19 \pm 0.83^{A,4,5}$	$8.87 \pm 0.35^{D,2,3}$
Day 7	$11.46 \pm 1.61^{B,3,4}$	$9.92 \pm 0.54^{C,4}$	$12.84 \pm 0.68^{A,5}$	$8.50 \pm 0.37^{D,2,3}$
Day 14	$11.39 \pm 1.68^{B,3,4}$	$10.14 \pm 0.53^{C,4}$	$13.40 \pm 0.80^{A,4,5}$	$8.19 \pm 0.34^{D,3}$
Day 21	$10.38 \pm 1.50^{B,3,4}$	$9.61 \pm 0.39^{C,4}$	$12.61 \pm 0.82^{A,5}$	$8.25 \pm 0.40^{D,3}$
Day 28	$9.88 \pm 1.18^{B,4}$	$9.39 \pm 0.40^{B,C,4}$	$11.45 \pm 3.54^{A,5}$	$8.21 \pm 0.44^{C,3}$

Different letters indicate statistically significant differences between rows, and different numbers indicate statistically significant differences between columns (A,1 = highest value) (p < 0.05).

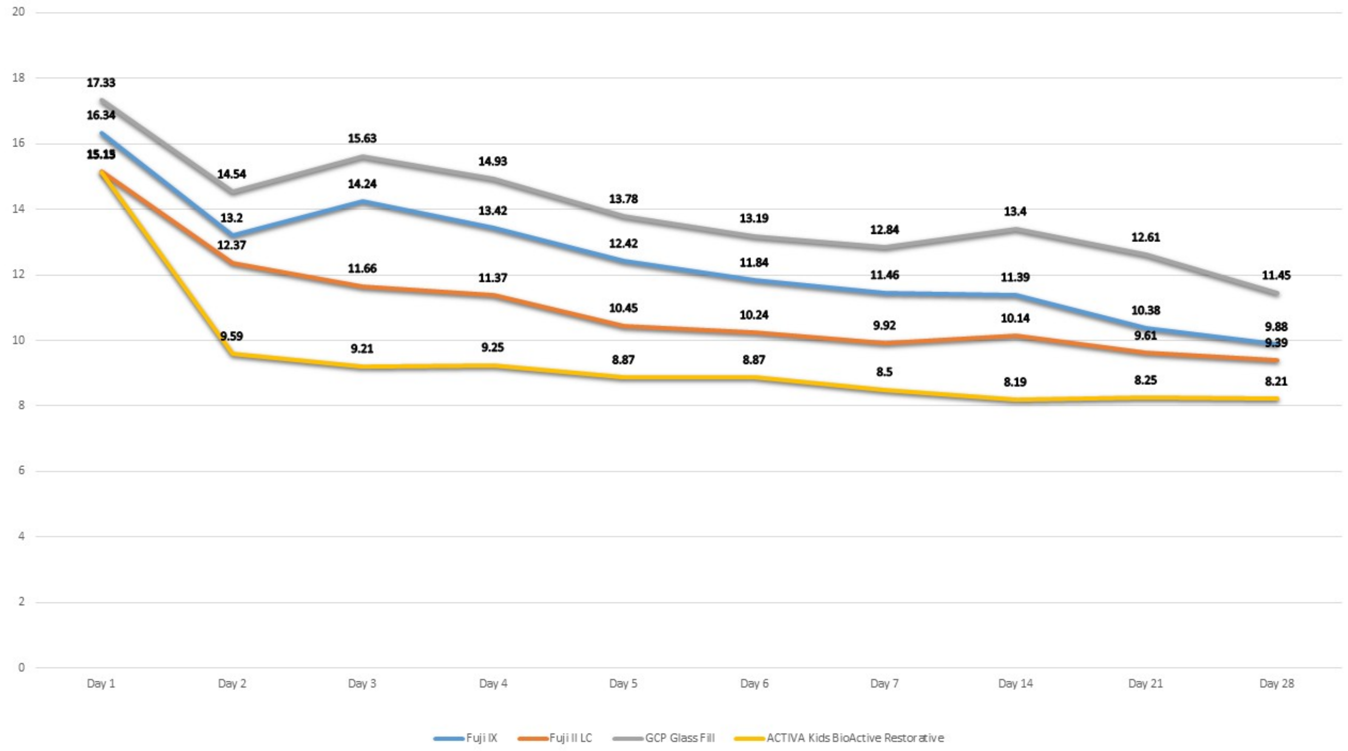

FIGURE 1. Mean daily fluoride release (ppm) of the tested restorative materials over the 28-day evaluation period. Error bars represent standard deviation. Statistical significance refers to within-material comparisons over time ($p < 0.05$).

materials in comparison with conventional and resin-modified glass ionomer cements.

The amount of fluoride released by restorative materials depends on multiple factors, including the fluoride ion content, chemical composition, and curing reaction of the material [6]. Consistent with previous studies [25, 34], the present study observed that glass carbomer exhibited higher fluoride release values compared with other restorative materials. This finding may be attributed to the higher fluoride content of glass carbomers relative to conventional glass ionomer cements [9, 39] and the presence of nanoparticles, which accelerate fluoride release [40]. Additionally, Bayrak *et al*. [34] reported that the heat generated during the polymerization reaction also increases fluoride release. In the present study, following glass carbomer, fluoride release values decreased sequentially for conventional glass ionomer cement, resin-modified glass ionomer cement, and ACTIVA™ Kids Bioactive Restorative material.

ACTIVA™ Kids Bioactive Restorative is a hybrid restorative material supplied in a self-mixing syringe and is theoretically suitable for bulk-fill application [41, 42]. During setting, a dual reaction mechanism occurs. In this process, an acid–base reaction takes place through the interaction of polyacrylic acid and dimethacrylate phosphate monomers with silanized fluoroaluminosilicate (FAS) glass fillers, resulting in the release of fluoride ions. The primary sources of fluoride in ACTIVA™ Kids Bioactive Restorative are sodium fluoride and the reactive glass components present in the filler phase. Concurrently with the acid–base reaction, chemically and light-activated resin polymerization occurs, contributing to the mechanical reinforcement of the material. Fluoride ions released as a result of the acid–base reaction may either undergo ion exchange with the surrounding environment or become retained within the polymer network through ionic bonding [42]. The lower fluoride release from ACTIVA™ Kids Bioactive Restorative material compared with conventional and resin-modified glass ionomer cements is consistent with previous studies [14, 15, 30]. This lower release is likely due to differences in the type and proportion of monomers in the material composition, as well as the presence of a resinous matrix that may limit ion mobility despite the inclusion of reactive glass fillers.

Studies on fluoride release from restorative materials generally report a high initial release on the first day, followed by a gradual decline over time [14, 23, 25, 34]. In agreement with these findings all materials in the present study exhibited a pronounced initial fluoride release, which subsequently decreased over the evaluation period. This phenomenon is primarily attributed to fluoride ions released from the superficial layers of the material [24, 43] and is commonly described as the "initial burst effect" [6, 43]. Bayrak *et al*. [34] and Kucukyilmaz *et al*. [38] similarly reported that glass carbomer exhibits a burst effect, consistent with the findings of the present study.

In this context, the significantly higher fluoride release observed with glass carbomer cements throughout the evaluation period indicates a distinct fluoride release profile when compared with conventional and bioactive restorative materials. However, it should be emphasized that fluoride release data obtained under *in vitro* conditions reflect material-related properties and do not directly translate into clinical performance. Factors such as oral environment, salivary dynamics, and patient-related variables may influence the clinical implications of fluoride release behavior.

Fluoride ion release has been shown to vary depending on the experimental storage conditions. In previous *in vitro* studies, various media, such as distilled water [44, 45], deionized water [34, 38, 45], and artificial saliva [46, 47], have been used to evaluate fluoride release from restorative materials. In the present study, deionized water was selected as the storage medium, as it has been the most commonly used medium in studies employing fluoride ion-selective electrodes [48]. Its lack of background ions allows standardized experimental conditions and facilitates accurate comparison of fluoride release patterns among different restorative materials. However, it should be acknowledged that this medium does not fully simulate the oral environment and may overestimate fluoride release compared with clinical conditions. Therefore, the results of the present *in vitro* study should be interpreted with caution, particularly when extrapolating to clinical performance.

Recent *in vitro* studies have demonstrated that environmental factors, such as pH and temperature, significantly influence ion release from bioactive restorative materials, with increased fluoride release reported under acidic conditions and elevated temperatures, particularly over extended storage periods [49–51]. In agreement with these findings, the present study also demonstrated a time-dependent fluoride release pattern, characterized by higher initial release followed by a gradual decrease. However, direct quantitative comparisons are limited due to methodological differences, as previous studies primarily investigated the effects of variable pH and temperature conditions, whereas the present study focused on comparing the intrinsic fluoride release capacities of restorative materials under standardized conditions.

Accordingly, the present findings provide complementary data by emphasizing material-dependent differences in fluoride release behavior, suggesting that both material composition and oral environmental factors are important determinants of fluoride availability in pediatric dentistry. From a clinical perspective, restorative materials capable of sustained fluoride release may be particularly beneficial for pediatric patients with a high risk of dental caries. Continuous fluoride availability can contribute to enamel remineralization and inhibition of demineralization, especially in children with limited oral hygiene compliance or frequent sugar intake. In this context, restorative materials that function as a long-term fluoride reservoir may support caries-preventive strategies in pediatric dentistry [6, 52]. Although the present study was conducted under *in vitro* conditions and does not fully replicate the oral environment, the comparative fluoride release profiles observed among the tested materials may provide clinically relevant information that could assist clinicians when considering restorative material options for pediatric patients with high caries risk.

The limitations of the present study should be acknowledged. First, the *in vitro* design cannot fully replicate the complex oral environment, where factors such as salivary flow, dietary challenges, masticatory forces, and biofilm activity may influence fluoride release dynamics. Second, deionized water was used as the storage medium, which may overestimate fluoride release compared with artificial saliva or clinical conditions, as ionic interactions and buffering capacity are absent. Third, pH cycling protocols and fluoride recharge procedures were not incorporated; therefore, the long-term fluoride release and recharge potential of the tested materials could not be evaluated. Finally, although standardized specimen preparation and measurement protocols were employed, the results should be interpreted with caution, and further *in situ* and clinical studies are required to confirm the clinical relevance of these findings in pediatric patients.

5. Conclusions

Within the limitations of this *in vitro* study, the tested restorative materials demonstrated distinct fluoride release profiles over the 28-day evaluation period. Glass carbomer and conventional glass ionomer cement exhibited higher fluoride release compared with resin-modified glass ionomer cement and ACTIVA™ Kids Bioactive Restorative under the experimental conditions applied. Although these findings suggest potential differences in fluoride release behavior among materials, they should be interpreted with caution, as *in vitro* conditions do not fully replicate the oral environment. Therefore, the results of the present study provide comparative experimental data, rather than definitive clinical recommendations.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request. All relevant data supporting the findings of this study are included within the article.

AUTHOR CONTRIBUTIONS

SA—conceived and designed the study, performed the experiments, analyzed the data, and drafted the manuscript. NT—provided scientific supervision, contributed to the interpretation of the data, and critically revised the manuscript. Both authors read and approved the final version of the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethics approval was not required for this *in vitro* study as no human or animal subjects were involved.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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