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# Exploring the potential of bioactive composites on the inhibition of enamel demineralization

Explorando o potencial dos compósitos bioativos na prevenção da desmineralização do esmalte

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## ABSTRACT

**Objective:** This work investigated the ion release from an experimental composite containing dicalcium phosphate dihydrate (DCPD) and a commercial composite containing ion-releasing particles, assessing their effectiveness in inhibiting enamel demineralization *in vitro*. **Material and Methods:** Ion release of calcium and phosphate was determined by inductively coupled plasma emission spectroscopy, while fluoride release was determined with an ion-specific electrode. Enamel specimens from bovine incisors were prepared measuring 6 x 6 mm and randomly distributed into three groups according to the restoration performed in their central portion into 3 groups (n=15): DCPD composite group (experimental composite); Giomer group (commercial composite Beautifil II®, Shofu), or a control group (Z250®, 3M ESPE). Samples underwent pH cycling in a remineralizing solution (20h) and a demineralizing solution (4h) for 8 days. Enamel surface hardness was evaluated at baseline (SH) and after the experiment (SH1), with transverse hardness measured post-pH cycling using a microhardness tester (25 g for 10 s). Surface hardness loss (%SHL), transverse hardness and integrated mineral loss (ΔKHN) were calculated. Data were analyzed by one-way ANOVA/Tukey test (alpha: 0.05). **Results:** DCDP group showed significantly higher calcium and phosphate concentrations, while Giomer group had higher fluoride, aluminum, boron, sodium, silicon and strontium concentrations compared to the control in both solutions. Giomer group demonstrated significantly less %SHL compared to Z250 group but was comparable to DCPD group. Only Giomer group effectively inhibited enamel surface demineralization, while both DCPD and Giomer group partially prevented subsurface demineralization. **Conclusion:** These findings suggest that incorporating DCPD into composites may enhance their protective effects against enamel demineralization, providing valuable insights for clinical applications in restorative dentistry.

## KEYWORDS

Bioactive materials; Composite resins; Demineralization; Dental caries; Remineralization.

## RESUMO

**Objetivo:** Este estudo investigou a liberação de íons a partir de dois compósitos dentais: um experimental, contendo fosfato de dicálcico dihidratado (DCPD) e um comercial, contendo partículas que liberam íons (Beautifil II®). O objetivo foi avaliar a eficácia desses compósitos na prevenção da desmineralização do esmalte *in vitro*. **Material e métodos:** A liberação de íons cálcio e fosfato foi quantificada por espectroscopia de emissão óptica por plasma acoplado indutivamente, enquanto a liberação de flúor foi medida com um eletrodo específico. Espécimes de esmalte provenientes de incisivos bovinos foram preparados com tamanho 6 x 6 mm e distribuídos aleatoriamente em três grupos de acordo com a restauração realizada em sua porção central (n=15): grupo DCPD (compósito experimental); grupo Giomer (compósito comercial) e grupo controle (resina Z250®, 3M ESPE).

As amostras foram submetidas à ciclagem de pH em uma solução remineralizante (20 h) e uma solução desmineralizante (4 h) durante 8 dias. A dureza da superfície do esmalte foi avaliada antes (SH) e após o experimento (SH1), utilizando-se um microdurômetro (25 g por 10 s). A perda da dureza da superfície (%PDS), dureza transversal e perda mineral integrada (ΔKHN) foram calculadas. Os dados foram analisados por ANOVA seguida do teste de Tukey ($\alpha = 0{,}05$). **Resultados:** O grupo DCPD apresentou concentrações significativamente superiores de cálcio e fosfato, enquanto o grupo Giomer liberou quantidades maiores de flúor, alumínio, boro, sódio, silício e estrôncio em comparação ao grupo controle, em ambas as soluções. O grupo Giomer demonstrou uma %PDS significativamente menor que o grupo Z250, mas semelhante ao grupo DCPD. Apenas o grupo Giomer inibiu efetivamente a desmineralização da superfície do esmalte, enquanto ambos os grupos DCPD e Giomer preveniram parcialmente a desmineralização subsuperficial. **Conclusão:** Esses achados sugerem que a incorporação de DCPD em compósitos pode potencializar seus efeitos protetores contra a desmineralização do esmalte, oferecendo informações valiosas para aplicações clínicas na odontologia restauradora.

## PALAVRAS-CHAVE

Materiais bioativos; Resina composta; Desmineralização; Cárie dentária; Remineralização.

## INTRODUCTION

Despite significant advancements in resin-based restorative materials, the longevity of composite restorations may be compromised, mainly due to the development of new caries lesions and restoration fractures [1]. Conventional dental composites and bonding agents are typically bioinert, serving only to replace lost tooth structure [2]. Consequently, there has been a focus on developing bioactive dental materials with remineralizing potential through ion release [3-8].

Resin composites that release fluoride, amorphous calcium phosphate (ACP), or that use bioactive adhesives aim to restore mineral content in affected dentin, potentially extending the restoration's lifespans [5]. Among ion-releasing materials, calcium phosphates (CaP) have been widely studied [4,9]. Other bioactive compounds include materials with calcium fluoride nanoparticles (nCaF2), ACP, nano-hydroxyapatite (nHA), and nano-fluorohydroxyapatite (nFHA) [10,11], as well as composites containing calcium silicates and bioactive glasses [12].

CaP has the advantage of biocompatibility, dissolving upon contact with living tissues and releasing calcium ($Ca^{2+}$) and hydrogen phosphate ($HPO_4^{2-}$) ions into the environment [13]. Experimental composites containing 40-80% (by weight) of various calcium phosphates, such as ACP, di-calcium phosphate anhydrous (DCPA), tetracalcium phosphate (TTCP), and DCPD, have shown to restore 14-71% of the mineral content in artificial caries lesions in enamel or dentin *in vitro* [2,14].

A recent class of bioactive materials, Giomers, introduced by Shofu (Kyoto, Japan) in the early 2000s, utilizes pre-reacted glass (PRG) technology. This technology incorporates fluorine-aluminum-silicate glass particles, pre-reacted with polyacrylic acid, dispersed in the resin. These PRG-based Giomers release ions such as aluminum, sodium, silicon, boron, strontium, and fluoride upon contact with biological fluids [15] potentially providing a synergistic effect in preventing caries lesions. While some studies suggest that restorations using Giomer technology exhibit clinical performance comparable to conventional composites [16,17] there remains a scarcity of studies focused on ion release from these materials [18-20]. Additionally, the clinical effectiveness of both experimental bioactive composites and Giomers warrants further investigation. Thus, the study aimed to evaluate ion release from an experimental composite containing DCPD particles and a commercial composite containing pre-reacted glass ionomer particles, compared to a control resin. We also sought to compare the inhibition of enamel demineralization by these composites *in vitro*. The null hypothesis tested was that ion release from bioactive composites does not prevent enamel demineralization.

## MATERIAL AND METHODS

### Synthesis of DCPD particles

Dicalcium phosphate dihydrate particles (DCPD, $CaHPO_4 \cdot 2H_2O$) were synthesized by precipitation, as previously described [9]. DCPD particles were synthesized through the stoichiometric reaction between ammonium phosphate, $(NH_4)_2HPO_4$, and calcium nitrate, $Ca(NO_3)_2 \cdot 4H_2O$. Two solutions of equal concentrations (0.078 mol/L) were prepared using distilled and deionized water. Using a peristaltic pump (9 mL/min), 400 mL of the calcium nitrate solution was added drop-wise to the same volume of ammonium phosphate solution, which previously received 7g of TEGDMA (2-methyl 2-propenic acid, Mw=286g/mol, ESSTECH, Technology Inc., Essington, PA). Precipitation occurred at room temperature under constant stirring, which was sustained for 30 min after dripping was finished. The pH of the final solution was 5.2. After particle decantation, they were rinsed in water to remove reminiscent ions and TEGDMA in excess. The resulting paste was freeze dried and a white powder was obtained.

### *Preparation of the experimental material containing DCPD*

An organic matrix based on BisGMA (2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy) phenyl]-propane, ESSTECH Technology Inc., Essington, PA) and TEGDMA (2-methyl 2-propenoic acid, ESSTECH) was mixed at a 1:1 mol ratio. The photoinitiators DMAEMA (2-(Dimethylamino) ethyl methacrylate) (Sigma-Aldrich Inc., St. Louis, MO, USA) and camphorquinone (Sigma-Aldrich Inc., Steinhein, Germany) were added at 0.5% by weight. A total of 30% by mass DCPD and 30% silanized barium glass were added to the resin matrix. The formulation of these resin was designed to closely match the total filler content of the commercial Giomer-based material. The final material consistency was also taken into account for particle calculation.

## Preparation of bovine enamel samples

Enamel blocks (6X6 mm) cut from the flattest area of bovine permanent incisors were prepared according to the technique described by Magalhães et al. [21]. Each enamel block was flattened with 320, 600 and 1200-grit sandpaper and then polished with felt discs with diamond pastes (Teclago, Vargem Grande Paulista, São Paulo, Brazil) in grits of 6, 3, and 1 $\mu$m.

The blocks were selected by surface hardness, considering a margin of 10% above or 10% below the average hardness value of all samples (279± 2.6 kg/mm$^2$) (n=15/group). Blocks were randomized into 3 groups. In the central part of the block, a 2-mm wide and 1.5-mm deep cavity was prepared with a self-limiting diamond tip (Drill 2294 KG Sorensen, São Paulo, Brazil) and then restored with one of the following composites: an experimental composite containing DCPD, a commercial ion-releasing composite (Beautifil II®, Shofu Dental GmbH, Ratingen, Germany), or a control composite (Z250®, 3M ESPE). The commercial ion-releasing composite is composed by fillers: s-PRG (aluminofluoro-borosilicate glass); Resin: BIS-GMA, TEGDMA and nano fillers 83.3 wt%. The control composite is composed by resin: BIS-GMA, UDMA and Bis-EMA and fillers: 60% (volume) silica/zirconia. The composition of the resin-based materials used in the study is summarized in Table I. No adhesive was applied to the cavity walls, in order to focus on the effects of composite without interference from adhesive, as described before [23].

Composites were inserted in the cavity in a single increment and light-cured for 20 seconds using an LED device (Radii-Cal, SDI, Bayswater, Australia) with an energy density of 48 J/cm$^2$ (1200 mW/cm$^2$ x 40 s).

**Table I -** Composition of the resin-based materials used in the study

| Experimental Group | Commercial Name / Type | Resin Matrix | Type of Filler | Filler Content (wt%) | Filler Composition |
|---|---|---|---|---|---|
| DCPD Group | Experimental (no trade name) | Bis-GMA + TEGDMA (1:1 molar ratio) | DCPD + silanized barium glass | 60% (30% DCPD + 30% Ba glass) | Dicalcium phosphate dihydrate $(CaHPO_4 \cdot 2H_2O)$;<br>silanized barium glass |
| Giomer Group | Beautifil II® (Shofu, Japan) | Bis-GMA + TEGDMA | s-PRG + nanofillers | 83.3% | Surface pre-reacted alumino-fluoro-borosilicate glass (s-PRG) |
| Z250 Group | Filtek Z250® (3M ESPE) | Bis-GMA + UDMA + Bis-EMA | Silica/zirconia | ~78%* | Inert silica/zirconia fillers |

*All filler contents are presented in weight percentage (wt%) to allow direct comparison. For the control composite (Filtek Z250®, 3M ESPE), the original filler load was described as 60% by volume in the manufacturer's datasheet. To ensure consistency with the other groups, this value was converted to approximately 78 wt% using established filler density ranges (2.0–2.6 g/cm$^3$) reported in the literature for silica/zirconia-based composites. Gonçalves et al. [22].

## Assessment of surface hardness

Surface hardness was evaluated using a microhardness tester (Shimadzu Corporation, Tokyo, Japan), consisting of a Knoop diamond indenter, with a load of 25 g applied for 10 s. The analysis was carried out using 3 indentations per specimen, 10 $\mu$m from the restoration margin and with distances of 100 $\mu$m between them, in the enamel region adjacent to the restorations, at the beginning (SH) and at the end of the experiment (SH1) (Figure 1). The percentage of surface loss was calculated according to the equation:

$$\%SHL = 100 \times \frac{(SH_1 - SH)}{SH} \quad (1)$$

## pH cycling

The blocks were then subjected to pH cycling for 8 days [24] that consisted of immersing the blocks in the demineralizing solution for 4h (0.05 mol/L acetate buffer, pH 5.0 and containing 1.28 mmol/L Ca, 0.74 mmol/L P and 0.03 mg F/mL) and in remineralizing solution (1.5 mmol/L Ca, 0.9 mmol/L P, 150 mmol/L KCl, 0.05 mg F/mL in 0.1 mol/L Tris buffer, pH 7.0) for 20 h at 37°C for 8 days. The proportions of demineralizing and remineralizing solutions per enamel area were 6.25 mL/mm$^2$ and 3.12 mL/mm$^2$, respectively. On the fourth day, the demineralizing and remineralizing solutions were replaced with new ones.

## Assessment of ion release

Aluminum, boron, strontium, silicon, and sodium were determined in samples of resin composite extracts by atomic absorption/emission spectrometry in flame and graphite furnace, using a SHIMADZU AA-6800 spectrometer, in both demineralizing and remineralizing solutions. The calibration of the spectrometer and mineralization of the samples were performed as described in Neves et al. [25] and Cavecci-Mendonça et al. [26]. Phosphorus was determined using the vanadomolybdophosphoric acid spectrophotometric method as described by Moraes et al. [27]. For the sample of the mineralization process, small adjustments were made: 10 mL of samples were transferred to 25-mL digestion flasks, and 2mL of 14 mol/L nitric acid and 0.50 mL of 30% hydrogen peroxide (m/m) were added to each flask. Subsequently, the mixture was heated in a digester block at 180°C for three hours. After cooling to room temperature, the acid extracts (final volume of approximately 6.50 mL) were transferred to 10-mL volumetric flasks and the volumes were completed with deionized water. All determinations were validated by analysis of certified reference material 1548a – Typical Diet – NIST.

Calcium and phosphate release were determined by inductively coupled plasma optical emission spectrometry (ICP-OES 700 series, Agilent Technologies, Santa Clara, CA, USA).

The concentration of F$^-$ ions was measured using the direct method. An F$^-$ ion-sensitive electrode (BN Model 9409, Orion, Cambridge, MA, USA) and a potentiometer (model 720 Orion, Cambridge, MA, USA) were used for analysis. F$^-$ readings were taken using 1 mL of each sample. Fluoride concentration ($\mu$g F/mL) was calculated by a standard correlation curve ($r^2 > 0.99$) based on the linear equation ($y = ax + b$). Samples were measured in duplicate and the average fluoride concentration of each sample was determined.

## Assessment of subsurface hardness

The integrated mineral loss was carried out at the end of the experiment using the average longitudinal/subsurface microhardness values (also done with a load of 25 g applied for 10 s) that are calculated at each internal distance (10, 30, 50, 70, 90, 110, 220, 330 $\mu$m), per sample

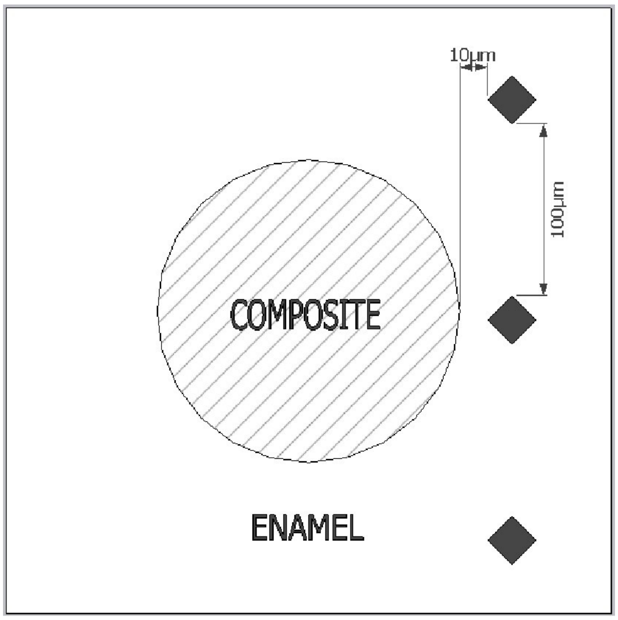

**Figure 1 -** Surface hardness indentations, 3 indentations per specimen, with distances of 100 µm between them, around the composite restoration.

**Table II -** Mean and standard deviation of ions release (mg/L) in the remineralizing solution (n=7)

| Remineralizing Solution | $PO_4^{2-}$ (mg/L) | Al (mg/L) | $SiO_4^{2-}$ (mg/L) | Sr (mg/L) | Ca (mg/L) |
|---|---|---|---|---|---|
| **DCPD group** | $32.8\pm3.2^a$ | $0.0108\pm1.7^b$ | $20.3\pm1.6^b$ | $0.0042\pm0.2^b$ | $9.17\pm2.5^a$ |
| **Giomer group** | $19.7\pm1.7^b$ | $0.0145\pm0.6^a$ | $28.6\pm0.7^a$ | $0.0067\pm0.4^a$ | $8.17\pm0.88^a$ |
| **Z250 group** | $19.4\pm2^b$ | $0.0098\pm0.8^b$ | $19.5\pm1.5^b$ | $0.0039\pm0.4^b$ | $4.3\pm0.85^b$ |

Values in the same column with different superscript letters differ significantly from each other (ANOVA and Tukey's test for individual comparisons; p<0.05).

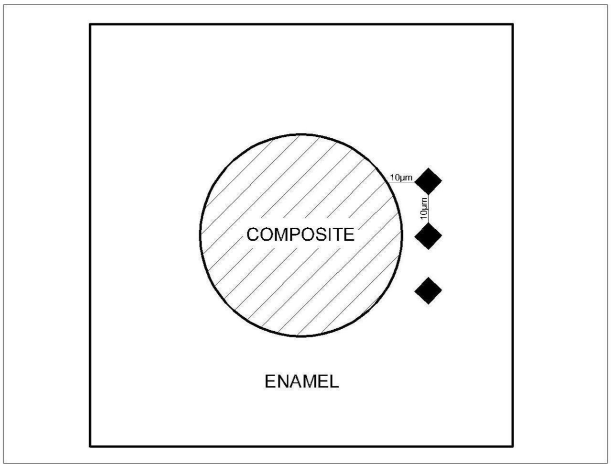

**Figure 2 -** Subsurface hardness indentations, at 10, 30, 50, 70, 90, 110, 220, 330 µm depths from the restoration margin.

(Figure 2). Indentation spacing was performed in the region adjacent to the restoration to evaluate the potential effect of the materials on the tissues surrounding the restoration. By averaging subsurface microhardness (KHN) per distance, ΔKHN (integrated mineral loss, KHN.$\mu$m) was calculated using the trapezoidal rule [28].

## STATISTICAL ANALYSIS

BioEstat software (5.3) was used for statistical analysis. The normality (Kolmogorov and Smirnov test) and homogeneity (Bartlett test) of the data were initially checked. The data were then analyzed using one-way ANOVA and the Tukey's test for individual comparisons. A 5% significance level was adopted in all tests.

## RESULTS

### Calcium, sodium, strontium, silicate, borate, aluminum and phosphate ions release

In the demineralizing solution, both Giomer and DCPD group had greater $Ca^{2+}$ concentration than Z250 group. DCDP group had significantly greater concentration compared to Giomer group. In the remineralizing solution, both DCPD and Giomer group had a higher concentration of $Ca^{2+}$ than Z250 group, not differing significantly from each other.

Giomer group had significantly higher $Na^+$ release compared to Z250 group, but it did not differ from DCPD group. In the remineralizing solution, Giomer group had significantly higher $Na^+$ release than Z250 and DCPD group. It is important to note, however, that DCPD group had a higher release of $Na^+$ in both the demineralizing and remineralization solutions compared to Z250 group.

Regarding Sr, $SiO_4^{2-}$ and $Al^{3+}$, a similar release pattern was observed: Giomer group had significantly higher release compared to Z250 and DCPD group, both in the demineralizing and remineralizing solutions. DCPD group was not significantly different from Z250 group in both the demineralizing and the remineralizing solutions.

As for $B_4O_7^{2-}$ ions, Giomer group had a significantly greater release than Z250 and DCPD group in the demineralizing solution, which did not differ from each other. In the remineralizing solution, the ion was not detected.

Finally, DCPD group had significantly greater concentration of $PO_4^{2-}$ than Z250 group and Giomer group in both solutions. Giomer group did not differ significantly from Z250 group in either the demineralization solution or the remineralization solution. The most important ion releases differences in remineralization solution are demonstrated in Table II and in the demineralization solution are demonstrated in Figure 3.

### Fluoride release

In the demineralizing solution, Giomer group had significantly higher fluoride release compared to Z250 group and did not differ from DCPD group. There was also no difference in fluoride release between DCPD and Z250 groups (Table III). Regarding the remineralizing solution, there were no significant differences among the groups.

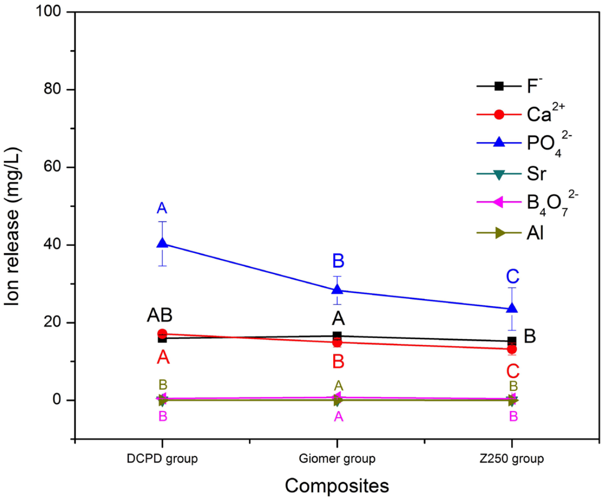

**Figure 3 -** Representative graph of ion release values in the demineralizing solution (n=7) (mg/L) $Ca^{2+}$ (17.11±0.7 mg/L) and $PO_4^{2-}$ (40.3±5.7 mg/L) in DCPD group was found in significantly higher amounts when compared to Giomer group ($Ca^{2+}$: 14.9±1.19 mg/L; $PO_4^{3-}$: 28.3±3.62 mg/L) and Z250 group ($Ca^{2+}$: 13.2±1.5 mg/L; $PO_4^{3}$: 23.5±5.5 mg/L). The demineralizing solution from Giomer group presented significantly more Al, B and Sr when compared to the solution from DCPD and Z250 groups. All three groups presented similar concentrations of fluoride, between 15,20 and 16,53 mg/L.

**Table III -** Mean and standard deviation of fluoride ion release (µg F/mL) in the demineralizing and remineralizing solutions (n=7)

| | Demineralizing solution (µg F/mL) | Remineralizing solution (µg F/mL) |
|---|---|---|
| **DCPD group** | 16.0 ± 0.9$^{ab}$ | 28.2 ± 1.0$^{a}$ |
| **Giomer group** | 16.5 ±1.0$^{a}$ | 27.4 ± 0.9$^{a}$ |
| **Z250 group** | 15.2 ± 0.9$^{b}$ | 27.2 ± 1.2$^{a}$ |

Values in the same column with different superscript letters differ significantly from each other (ANOVA and Tukey's test for individual comparisons; p<0.05).

## Surface hardness loss (%SHL), subsurface hardness and integrated mineral loss (ΔKHN)

One-way analysis of variance (ANOVA) showed a significant difference between treatments regarding to the percentage of microhardness surface loss (%SHL) ($p<0.05$), in which Giomer group differed significantly Z250 group, but without significant difference between DCPD group, which was also unable to differ from Z250 group. Table IV shows the mean and standard deviation of the initial surface hardness ($SH_1$), SH after pH cycling ($SH_2$) and percentage of surface hardness loss (%SHL). It is worth noting that there was no significant difference among groups in relation to initial superficial hardness (Table IV).

Regarding subsurface hardness, when comparing the same material in different depths adjacent to the restorations (10, 30 and 50 $\mu$m) there were significant differences among the demineralization pattern, where the control resin was not able to prevent subsurface demineralization (there were no significant differences among the depths) while DCPD and Giomer groups presented significant differences among the depths (ANOVA and Tukey's test for individual comparisons; $p<0.05$) (Table V).

However, in relation to the integrated mineral loss, calculated through the longitudinal hardness of the studied groups, the one-way analysis of variance (ANOVA) did not detect significant differences between the groups ($p> 0.05$) (Table VI).

**Table IV -** Mean and standard deviation of the initial surface hardness, SH after pH cycling (SH2) and percentage of surface hardness loss (%SHL) (n=15)

| Experimental Groups | Initial hardness ($SH_1$)* | After pH cycling ($SH_2$) | Hardness loss %SHL* |
|---|---|---|---|
| DCPD group | 291±83$^{a}$ | 265±83 | -7.64±20$^{ab}$ |
| Giomer group | 261±77$^{a}$ | 250±89 | -2.35±26$^{b}$ |
| Z250 group | 308±71$^{a}$ | 218±120 | -30.39±38$^{a}$ |

*Values in the same column with different superscript letters differ significantly from each other (ANOVA and Tukey's test for individual comparisons; p<0.05).

**Table V -** Mean and standard deviation of the subsurface hardness in depths adjacent to the restorations of the experimental groups (n=15)

| Experimental Groups | Mean subsurface hardness (10 µm) | Mean subsurface hardness (30 µm) | Mean subsurface hardness (50 µm) |
|---|---|---|---|
| DCPD group | 183.3±47.29$^{a}$ | 214.7±37.33$^{ac}$ | 235.6±26.23$^{c}$ |
| Giomer group | 196.3±32.06$^{a}$ | 226.00±25.55$^{b}$ | 226.2±37.45$^{b}$ |
| Z250 group | 163.3±45.94$^{a}$ | 180.4±55.96$^{a}$ | 176.1±54.23$^{a}$ |

Values in the same line with different superscript letters differ significantly from each other (ANOVA and Tukey's test for individual comparisons; p<0.05).

**Table VI -** Mean and standard deviation of the integrated mineral loss (△KHN), calculated through subsurface microhardness values of the experimental groups (n=15)

| Experimental Groups | Mean subsurface hardness | Integrated mineral loss (∆KHN) |
|---|---|---|
| DCPD group | 238.9±49.2 | 6010±2649[a] |
| Giomer group | 229.8±52.8 | 5945±2633[a] |
| Z250 group | 182.0±60.3 | 7346±4132[a] |
| p-value* | | 0.569 |

*Values in the same column with different superscript letters differ significantly from each other (ANOVA (p>0.05)).

## DISCUSSION

The present study demonstrated that both the experimental DCPD-based composite and the Giomer-based composite exhibited superior ion release compared to the control composite (Z250). Notably, the DCPD group showed significantly higher release of calcium and phosphate ions, while the Giomer group presented elevated concentrations of fluoride, aluminum, boron, sodium, silicon, and strontium ions. Furthermore, the Giomer group effectively inhibited enamel surface demineralization, while both bioactive composites partially prevented subsurface demineralization. These findings underscore the potential of incorporating ion-releasing particles, to enhance restorative materials protective effects against caries-related demineralization.

The clinical management of carious lesions has been extensively studied [29,30]. Advances in ion-releasing resin-based materials and bioactive adhesives aim to extend restoration longevity by facilitating mineral redeposition [2,5,31]. Among these innovations, the incorporation of calcium orthophosphates (CaP) into resin-based restorative materials has shown promise in reducing the risk of caries at the tooth-restoration interface [4].

Dicalcium phosphate dihydrate (DCPD) offers intermediate solubility among CaP phases, enabling greater ion release compared to less soluble CaP forms. Additionally, its refractive index (1.54–1.55), similar to the resin matrix (1.50–1.55), enhances light transmission during curing, making it a promising restorative material [4].

The findings of this study provide insight into the behavior of bioactive materials in different pH environments. Bioactive materials exhibited higher overall ion release in the demineralizing solution compared to the remineralizing solution, except for fluoride, which demonstrated a contrasting pattern, being more concentrated in the remineralizing solution. These results emphasize the dynamic interaction of bioactive materials with their environment and their potential for site-specific ion delivery.

The DCPD-based composite showed significantly greater release of calcium and phosphate ions compared to the Giomer and Z250 groups in the demineralizing solution. This behavior highlights the material's ability to release ions under acidic conditions, which are often associated with caries activity. Interestingly, calcium ion concentrations in the remineralizing solution were comparable between the DCPD and Z250 groups, suggesting a mechanism of redeposition and mineral formation on the enamel surface during pH cycling. This phenomenon likely reflects the material's capacity to contribute to remineralization under favorable conditions.

Phosphorus ion release exhibited a similar trend, with the DCPD group consistently outperforming the other groups in both solutions. The higher phosphorus-to-calcium ratio observed may indicate precipitation processes, reinforcing the idea that these ions are actively participating in mineral redeposition. These findings underline the potential of DCPD-based composites to enhance the availability of critical ions for enamel repair.

The resin matrix composition also plays a key role in ion release from composite materials. This is mainly influenced by the hydrophilic or hydrophobic nature of the monomers and their capacity for water sorption. Bis-GMA is viscous and relatively hydrophobic due to its aromatic structure, which limits water diffusion through the polymer network [32]. In contrast, TEGDMA is a low-viscosity, hydrophilic monomer that increases water uptake and facilitates ion mobility [22]. In the experimental DCPD composite, the 1:1 Bis-GMA/TEGDMA ratio likely enhanced water sorption, promoting the diffusion of calcium and phosphate ions. This proportion was also shown to provide an optimal balance between mechanical performance and polymerization stress in experimental composites, combining favorable ion transport characteristics with adequate structural integrity [33].

The Giomer-based composite, on the other hand, demonstrated elevated release of fluoride, aluminum, boron, sodium, silicon, and strontium ions in the demineralizing solution. This aligns with its design as a fluoride-releasing material, which has been associated with caries prevention and enamel strengthening. The sustained fluoride release in the remineralizing solution further supports the material's role in promoting long-term enamel protection, contrasting with the rapid initial release typically seen in glass ionomer cements [18].

The unique fluoride release pattern observed in this study is consistent with prior research, which suggests that fluoride consumption during fluorapatite formation under acidic conditions contributes to its reduced concentration in the demineralizing solution. This mechanism highlights the dual role of fluoride in neutralizing acidic environments and reinforcing the enamel structure. The slow, sustained fluoride release noted in bioactive resins offers a distinct advantage over materials that exhibit an abrupt release followed by stabilization, as it may provide prolonged protection against demineralization [18,34,35]. Furthermore, in the study by Naoum et al. [35] the recharge potential of bioactive resins was measured using 18-month-old specimens, and it was found that a giomer (Beautifil II) had a comparable long-term fluoride release pattern after recharge treatment as a GIC material (Fuji IX Extra). In another study, the giomer showed a higher recharge potential compared to other fluoride-containing composite materials [36].

Kaga et al. [37] highlighted the ability of bioactive materials, such as Giomer composites, to neutralize acidic pH and suppress caries progression. Their findings revealed high concentrations of calcium and phosphorus ions in all tested materials, with the Giomer composite additionally releasing aluminum, fluoride, sodium, silicon, and strontium ions. These results align with the present study, reinforcing the capacity of Giomer-based materials to actively contribute to enamel protection through ion release. The Giomer composite also demonstrated superior performance in maintaining enamel surface hardness, as evidenced by significantly lower surface hardness loss (%SHL) compared to the Z250 group. This behavior is likely due to the formation of a supersaturated fluorapatite layer on the enamel surface, which can inhibit deeper ion penetration. Such a protective mechanism has been reported in other studies on fluoride-releasing materials [18,38] and helps to explain the enhanced surface protection observed in the Giomer group. Subsurface protection, however, varied among the materials. Consistent with Pinto et al. [39], the DCPD composite demonstrated reduced subsurface mineral loss compared to the Z250 group. Both the DCPD and Giomer groups exhibited significant differences in demineralization patterns at various depths (10, 30, and 50 $\mu$m), highlighting their ability to prevent subsurface demineralization. Despite these promising results, integrated mineral loss, assessed through longitudinal hardness, did not differ significantly among groups, suggesting that the protective effects of these materials may be depth-dependent and influenced by their ion release profiles.

While the findings are encouraging, further validation is needed using more clinically relevant models, such as *in situ* or microcosm biofilm studies. The limitations of *in vitro* pH cycling models, such as their closed nature and restricted mineral exchange, may result in supersaturation and the formation of resistant surface layers that limit deeper ion penetration. Furthermore, remineralization or the prevention of subsurface demineralization of *in vitro* pH cycling models is commonly observed up to around 100 micrometers in longitudinal hardness analysis, which may not correspond to the complete remineralization of the white spot in a clinical of biofilm model situation. Future research should aim to replicate real-world clinical conditions to better understand the long-term benefits of bioactive composites in restorative dentistry.

## CONCLUSION

It was concluded that both bioactive composites exhibited significantly higher ion release compared to the control composite (Z250). Notably, while the Giomer composite effectively inhibited enamel surface demineralization, both the DCPD and Giomer composites provided partial protection against subsurface demineralization. These findings underscore the potential of ion-releasing particles to enhance the protective effects of restorative materials against caries-related demineralization.

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## Author's Contributions

MCR, CABC: Conceptualization. MCR, CABC: Data Curation. CABC: Funding Acquisition. PAN, RPOG, SAPA, EAS: Investigation. PAN, RPOG, SAPA, EAS, MCR, CABC: Methodology. CABC: Project Administration. PAN, RPOG, MCR, CABC: Resources. MCR: Software. CABC: Supervision. CABC: Visualization. PAN, RPOG, CABC: Writing – Original Draft Preparation. MCR, RRB, CABC: Writing – Review & Editing.

## Conflict of Interest

No conflicts of interest declared concerning the publication of this article.

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## Regulatory Statement

The Normative Resolution CONCEA (National Council for the Control of Animal Experimentation) No. 55/2022, establishes in its Article 3: "Projects that exclusively use materials or products of animal origin from legally constituted commercial establishments duly inspected by the competent authorities are exempt from submission to the Ethics Committees for the Use of Animals."

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