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# Topographic and Elemental Evaluation of a Bioactive Caries Detecting Dye Compared with CPP-ACP and Acidulated Phosphate Fluoride on Demineralised Enamel and Dentin

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## Abstract

# Background

Conventional remineralising agents such as Casein Phosphopeptide–Amorphous Calcium Phosphate (CPP-ACP) and Acidulated Phosphate Fluoride (APF) have demonstrated the ability to enhance mineral deposition and improve resistance to acid attack. Recently, a Bioactive Caries Detecting (BCD) dye containing nano-hydroxyapatite and chitosan has been introduced with the potential to combine caries detection with therapeutic remineralisation. However, scientific evidence evaluating its remineralising efficacy in comparison with established agents remains limited. This study aimed to evaluate and compare the remineralising potential of BCD dye, CPP-ACP, and APF on demineralised enamel and dentin using Field Emission Scanning Electron Microscopy (FESEM) and Energy Dispersive Spectroscopy (EDS).

# Methods

Forty orthodontically extracted human premolars were sectioned to obtain standardized enamel and dentin specimens. Artificial demineralisation was induced using 35% phosphoric acid. Specimens were randomly allocated into four groups: CPP-ACP paste, BCD dye solution, APF gel (1.23%), and untreated control. Each material was applied for three consecutive days, and samples were stored in artificial saliva between applications. Surface morphological changes were evaluated using FESEM, while elemental composition including calcium and phosphorus levels, was quantified using EDS. A statistical analysis was conducted using one-way ANOVA, with a significance level set at $p < 0.05$.

# Results

FESEM analysis demonstrated dense mineral deposition in enamel and near-complete dentinal tubule occlusion in the BCD group. CPP-ACP and APF groups exhibited comparatively scattered crystalline deposits. EDS analysis revealed significantly higher calcium and phosphorus levels in enamel specimens treated with APF and BCD compared with CPP-ACP ($p < 0.001$). In dentin specimens, mineral preservation was greater in the CPP-ACP and BCD groups, whereas APF showed comparatively lower calcium values.

# Conclusion

The bioactive caries detecting dye demonstrated consistent remineralising activity across both enamel and dentin substrates and promoted effective dentinal tubule occlusion. These findings suggest that BCD may be a promising, minimally invasive material that combines diagnostic and therapeutic benefits for caries management.

## Introduction

Dental caries remains one of the most prevalent chronic diseases affecting individuals worldwide. It is a multifactorial process resulting from the interaction between cariogenic microorganisms, fermentable

carbohydrates, and susceptible tooth surfaces. Bacterial species such as Streptococcus mutans and Lactobacillus metabolize dietary sugars to produce organic acids, which reduce the pH of the dental biofilm below the critical threshold of approximately 5.5. This acidic environment promotes dissolution of hydroxyapatite crystals, leading to progressive demineralisation of enamel and dentin. If the process continues unchecked, it may eventually result in cavitation and structural destruction of the tooth [1].

Contemporary approaches in caries management emphasize early diagnosis and non-invasive or minimally invasive treatment strategies. Remineralisation represents the natural or therapeutically enhanced redeposition of calcium and phosphate ions into partially demineralised dental tissues. By restoring mineral content and reinforcing the crystalline structure of enamel, remineralisation can arrest or reverse early carious lesions and reduce the need for operative interventions. Consequently, several preventive and therapeutic agents have been developed to promote remineralisation and improve resistance of dental tissues to acidic challenges [2].

Casein Phosphopeptide–Amorphous Calcium Phosphate (CPP-ACP) has gained considerable attention due to its ability to stabilize calcium and phosphate ions in a bioavailable amorphous form. CPP-ACP complexes adhere to dental surfaces and plaque, maintaining a supersaturated environment of calcium and phosphate ions that facilitates diffusion into subsurface enamel lesions and enhances remineralisation [3, 4]. Fluoride-based therapies remain another cornerstone of preventive dentistry. Acidulated Phosphate Fluoride (APF) gel, containing 1.23% fluoride ions, enhances enamel resistance by promoting the formation of fluorapatite crystals and increasing fluoride uptake following mild enamel surface etching [5, 6].

Recently, a novel Bioactive Caries Detecting (BCD) dye has been introduced with the aim of combining diagnostic caries detection with therapeutic remineralisation. Unlike conventional caries detecting dyes that primarily differentiate infected dentin, this formulation contains nano-hydroxyapatite, chitosan, iobitridol, and a dye component. Nano-hydroxyapatite has been shown to promote mineral deposition, enhance enamel remineralisation, and facilitate dentinal tubule occlusion, whereas chitosan may improve adhesion and interaction with the dental substrate [7–9]. Advanced analytical techniques are essential for evaluating remineralisation at both structural and elemental levels. Field Emission Scanning Electron Microscopy (FESEM) enables high-resolution visualization of surface morphology and mineral deposition patterns, while Energy Dispersive Spectroscopy (EDS) allows quantitative analysis of elemental composition such as calcium and phosphorus within dental tissues [10, 11].

Although CPP-ACP and APF have been extensively studied as remineralising agents, evidence regarding the remineralising efficacy of Bioactive Caries Detecting dye remains limited. Considering its novel formulation and potential dual diagnostic–therapeutic function, a comparative evaluation with established remineralising agents is warranted. Therefore, the present study aimed to assess and compare the remineralising potential of BCD dye, CPP-ACP, and Acidulated Phosphate Fluoride on demineralised enamel and dentin using FESEM and EDS analysis.

## Materials and Methods

**Study design and ethical approval**: The present in vitro experimental study was conducted in the Department of Conservative Dentistry and Endodontics after obtaining approval from the Institutional Ethics Committee (Ref. No: IEC-IDS/IDS/SOA/2024/I-03). The study was designed to evaluate and compare the remineralising efficacy

of three different agents on artificially demineralised enamel and dentin using morphological and elemental analytical techniques.

### Pilot study

Prior to the main experiment, a pilot study was performed on 12 extracted human premolars to standardize the experimental protocol and evaluate the feasibility of the procedures. The pilot study aimed to refine methodological parameters including the demineralisation protocol, remineralising agent application procedures, and analytical evaluation using Field Emission Scanning Electron Microscopy (FESEM) and Energy Dispersive Spectroscopy (EDS). This preliminary investigation helped ensure reproducibility and methodological consistency. Internal reliability of the protocol was assessed using Cronbach's alpha, which demonstrated good reliability ($\alpha = 0.85$).

### Sample source and sample size calculation

Orthodontically extracted human premolars were collected from the Department of Oral and Maxillofacial Surgery. The sample size was determined using G*Power software (version 3.1.9.7) with a significance level of 5% ($p = 0.05$), statistical power of 80%, and an effect size (f) of 0.28. Based on this calculation, a total of 40 specimens were required for the study. Twelve samples (6 in each for enamel and dentin groups) were allocated to each of the experimental groups, and four specimens (two each for enamel and dentin) served as the control group, providing an actual statistical power of 0.813.

## Inclusion and exclusion criteria

The study included non-carious orthodontically extracted permanent premolars with intact crown structure. Teeth presenting with caries, fractures, cracks, fluorosis, or previous endodontic treatment were excluded. Primary teeth and teeth with structural defects were also excluded to ensure uniformity of the samples.

### Specimen preparation

The collected teeth were initially cleaned and immersed in 2.5% sodium hypochlorite solution for 15 minutes to remove organic debris and soft tissue remnants. Following disinfection, the teeth were rinsed thoroughly and stored in normal saline until use. Each tooth was examined under a stereomicroscope to confirm the absence of cracks or structural defects. For enamel specimens, twenty teeth were sectioned using a diamond disc under continuous water cooling to obtain enamel blocks measuring 3 × 3 × 1 mm from the buccal surface. For dentin specimens, twenty teeth were sectioned to obtain superficial dentin blocks measuring 3 × 3 × 2 mm. All prepared specimens were subjected to artificial demineralisation by applying 35% phosphoric acid gel for 120 seconds. The specimens were then thoroughly rinsed with distilled water and stored in artificial saliva for 24 hours prior to treatment.

### Materials used

Enamel and dentin etching was performed using GLUMA® Etch 35 Gel (Kulzer, Hanau, Germany) containing 35% orthophosphoric acid. Artificial saliva used for specimen storage was Artificial Saliva for Medical and Dental Research (Nanochemazone® Inc./Aritech Chemazone Pvt Ltd, Kurukshetra, Haryana, India) with a pH of 6.8, containing sodium carboxymethyl cellulose to simulate the viscosity of natural saliva [11, 12]. The fluoride-

based remineralising agent used was FLUOROTOP APF gel (1.23% fluoride ion; Prevest DenPro Limited, Jammu, India), which also contains xylitol as an anticaries component. The CPP-ACP based remineralising agent used was GC Tooth Mousse (GC Corporation, Tokyo, Japan) containing Recaldent™ (Casein Phosphopeptide–Amorphous Calcium Phosphate) an novel BCD was developed by our research team [7]

**Group allocation and treatment protocol**

Following specimen preparation and demineralisation, the samples were randomly allocated into four experimental groups using a simple randomisation method. Group 1(each n = 6 for enamel and dentin) specimens were treated with CPP-ACP paste (Recaldent®, GC Tooth Mousse, GC Corporation, Tokyo, Japan). The paste was applied to the specimen surface using a microbrush and allowed to remain in contact for four minutes, after which the specimens were rinsed with distilled water. Group 2 specimens (enamel and dentin) received treatment with the Bioactive Caries Detecting (BCD) dye solution, which was applied by gently scrubbing the specimen surface using a microbrush for 40 seconds, followed by thorough rinsing with water. Group 3 specimens (enamel and dentin) were treated with 1.23% Acidulated Phosphate Fluoride (APF) gel (FLUOROTOP APF; Prevest DenPro Limited, Jammu, India). The gel was applied to the specimen surface for four minutes and subsequently rinsed. Group 4 served as the control group, in which specimens (enamel and dentin) underwent the demineralisation protocol but did not receive any remineralising treatment. All experimental applications were performed for three consecutive days, and between each application, the specimens were stored in artificial saliva to simulate oral environmental conditions and maintain specimen hydration.

**FESEM and EDS Analysis**

After completion of the treatment procedures, all specimens were prepared for microscopic and elemental evaluation to assess the surface characteristics and mineral composition following remineralisation. Initially, the samples were dehydrated by placing them in desiccators to eliminate moisture that could interfere with electron microscopy imaging. Subsequently, the specimens were sputter-coated with a thin layer of gold using a vacuum evaporator (EIKO IB-3 ion coater, Hitachi). Gold coating enhances electrical conductivity and prevents surface charging during electron-beam exposure, thereby improving the clarity and resolution of microscopic images. The treated specimens were then examined using Field Emission Scanning Electron Microscopy (FESEM; JEOL JSM-IT800) at different magnifications. FESEM is an advanced imaging technique that uses a focused electron beam to generate high-resolution images of the specimen surface. This technique enables detailed visualization of ultrastructural changes on enamel and dentin surfaces, including mineral deposition patterns, surface morphology, and the extent of dentinal tubule occlusion. In the present study, FESEM analysis was used to qualitatively evaluate the morphological changes produced by the different remineralising agents and to identify variations in mineral deposition across the experimental groups.

To complement the morphological evaluation, Energy Dispersive X-ray Spectroscopy (EDS; EDAX-AMETEK, Model ELEMENT-E2360) was performed on the same specimens. EDS is an analytical technique integrated with scanning electron microscopy that allows quantitative determination of elemental composition within a sample. When the electron beam interacts with the specimen surface, characteristic X-rays are emitted by the constituent elements. These X-rays are detected and analysed to determine the elemental distribution present in the sample. In the present study, EDS was used to quantify the weight percentage of elements, including

oxygen, sodium, phosphorus, chlorine, calcium, potassium, and carbon. Calcium and phosphorus levels were particularly evaluated as indicators of mineral deposition and the tested agents' remineralisation potential.

# Statistical Analysis

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software version 27. Prior to the comparative analysis, the distribution of the collected data was assessed to determine whether it followed a normal distribution. The Shapiro–Wilk test was used to evaluate data normality, and the results confirmed that the data were normally distributed across the experimental groups ($p > 0.05$). Descriptive statistical analysis was subsequently performed to summarize the elemental values obtained from EDS measurements. The results were expressed as mean ± standard deviation, providing estimates of central tendency and variability within each experimental group.

To compare elemental composition differences among the experimental groups, a one-way Analysis of Variance (ANOVA) was performed. This statistical test allows comparison of the mean values of more than two independent groups and determines whether the observed differences are statistically significant. The ANOVA analysis helped identify variations in mineral deposition patterns among the CPP-ACP, BCD, APF, and control groups. A p-value less than 0.05 was considered statistically significant, indicating that the observed differences between groups were unlikely to have occurred by chance.

# Results

The elemental analysis performed using Energy Dispersive X-ray Spectroscopy (EDS) revealed distinct remineralisation patterns among the different treatment groups. Quantitative comparison of elemental composition demonstrated statistically significant variations in mineral deposition following treatment with CPP-ACP, Bioactive Caries Detecting (BCD) dye, and Acidulated Phosphate Fluoride (APF) as discussed in Table 1 & Table 2

Table 1
Analysing the elemental composition of enamel in the CPP-ACP, BCD, and APF groups.

| Element | Group | N | Mean | SD | SE | 95% CI Lower | 95% CI Upper | F | p-value |
|---|---|---|---|---|---|---|---|---|---|
| **CaK** | CPP-ACP | 6 | 24.5000 | 1.18322 | 0.48305 | 23.2583 | 25.7417 | 234.019 | **0.000*** |
| | BCD | 6 | 34.8000 | 0.54037 | 0.22061 | 34.2329 | 35.3671 | | |
| | APF | 6 | 35.2833 | 1.07781 | 0.44001 | 34.1522 | 36.4144 | | |
| **OK** | CPP-ACP | 6 | 11.7500 | 0.50498 | 0.20616 | 11.2201 | 12.2799 | 284.954 | **0.000*** |
| | BCD | 6 | 22.6833 | 0.66156 | 0.27008 | 21.9891 | 23.3776 | | |
| | APF | 6 | 27.7667 | 1.88113 | 0.76797 | 25.7925 | 29.7408 | | |
| **CK** | CPP-ACP | 6 | 23.3000 | 0.72111 | 0.29439 | 22.5432 | 24.0568 | 3650.745 | **0.000*** |
| | BCD | 6 | 1.6167 | 0.27869 | 0.11377 | 1.3242 | 1.9091 | | |
| | APF | 6 | 1.8833 | 0.40702 | 0.16617 | 1.4562 | 2.3105 | | |
| **PK** | CPP-ACP | 6 | 3.2833 | 0.40208 | 0.16415 | 2.8614 | 3.7053 | 1040.713 | **0.000*** |
| | BCD | 6 | 13.1667 | 0.46332 | 0.18915 | 12.6804 | 13.6529 | | |
| | APF | 6 | 13.6333 | 0.46332 | 0.18915 | 13.1471 | 14.1196 | | |
| **NaK** | CPP-ACP | 6 | 3.7000 | 0.31623 | 0.12910 | 3.3681 | 4.0319 | 185.116 | **0.000*** |
| | BCD | 6 | 0.2500 | 0.33317 | 0.13601 | -0.0996 | 0.5996 | | |
| | APF | 6 | 1.3500 | 0.30166 | 0.12315 | 1.0334 | 1.6666 | | |
| **ZrL** | CPP-ACP | 6 | 23.6167 | 0.61128 | 0.24956 | 22.9752 | 24.2582 | 1402.681 | **0.000*** |
| | BCD | 6 | 7.4167 | 0.42622 | 0.17401 | 6.9694 | 7.8640 | | |
| | APF | 6 | 6.8667 | 0.77889 | 0.31798 | 6.0493 | 7.6841 | | |
| **ZnL** | CPP-ACP | 6 | 0.0000 | 0.00000 | 0.00000 | 0.0000 | 0.0000 | 143.289 | **0.000*** |
| | BCD | 6 | 3.5833 | 0.73326 | 0.29935 | 2.8138 | 4.3528 | | |
| | APF | 6 | 0.0000 | 0.00000 | 0.00000 | 0.0000 | 0.0000 | | |
| **ClK** | CPP-ACP | 6 | 0.0000 | 0.00000 | 0.00000 | 0.0000 | 0.0000 | 4.372 | **0.032*** |
| | BCD | 6 | 0.3833 | 0.44907 | 0.18333 | -0.0879 | 0.8546 | | |

*Statistically significant

| Element | Group | N | Mean | SD | SE | 95% CI Lower | 95% CI Upper | F | p-value |
|---|---|---|---|---|---|---|---|---|---|
| | APF | 6 | 0.0000 | 0.00000 | 0.00000 | 0.0000 | 0.0000 | | |
| **MgK** | CPP-ACP | 6 | 0.0000 | 0.00000 | 0.00000 | 0.0000 | 0.0000 | 19.027 | **0.000*** |
| | BCD | 6 | 0.0000 | 0.00000 | 0.00000 | 0.0000 | 0.0000 | | |
| | APF | 6 | 0.4833 | 0.27142 | 0.11081 | 0.1985 | 0.7682 | | |
| *Statistically significant | | | | | | | | | |

Table 2
Analysing the elemental composition of dentin in the CPP-ACP, BCD, and APF groups.

| Element | Group | N | Mean | SD | SE | 95% CI Lower | 95% CI Upper | F | p-value |
|---|---|---|---|---|---|---|---|---|---|
| **CaK** | CPP-ACP | 6 | 20.2833 | 0.94956 | 0.38766 | 19.2868 | 21.2798 | 1733.675 | **0.000*** |
| | BCD | 6 | 20.7333 | 0.43665 | 0.17826 | 20.2751 | 21.1916 | | |
| | APF | 6 | 1.6500 | 0.37283 | 0.15221 | 1.2587 | 2.0413 | | |
| **OK** | CPP-ACP | 6 | 15.6167 | 0.43089 | 0.17591 | 15.1645 | 16.0689 | 677.579 | **0.000*** |
| | BCD | 6 | 30.6000 | 0.34059 | 0.13904 | 30.2426 | 30.9574 | | |
| | APF | 6 | 27.7000 | 1.17303 | 0.47889 | 26.4690 | 28.9310 | | |
| **CK** | CPP-ACP | 6 | 16.0000 | 0.74565 | 0.30441 | 15.2175 | 16.7825 | 438.661 | **0.000*** |
| | BCD | 6 | 14.7667 | 0.35024 | 0.14298 | 14.3991 | 15.1342 | | |
| | APF | 6 | 35.6500 | 2.22598 | 0.90875 | 33.3140 | 37.9860 | | |
| **PK** | CPP-ACP | 6 | 3.1500 | 0.27386 | 0.11180 | 2.8626 | 3.4374 | 519.206 | **0.000*** |
| | BCD | 6 | 8.0333 | 0.33267 | 0.13581 | 7.6842 | 8.3824 | | |
| | APF | 6 | 0.8333 | 0.53166 | 0.21705 | 0.2754 | 1.3913 | | |
| **NaK** | CPP-ACP | 6 | 5.2333 | 0.38816 | 0.15846 | 4.8260 | 5.6407 | 287.597 | **0.000*** |
| | BCD | 6 | 0.1167 | 0.20412 | 0.08333 | -0.0975 | 0.3309 | | |
| | APF | 6 | 0.5000 | 0.56214 | 0.22949 | -0.0899 | 1.0899 | | |
| **ZrL** | CPP-ACP | 6 | 18.7667 | 0.70333 | 0.28713 | 18.0286 | 19.5048 | 1734.572 | **0.000*** |
| | BCD | 6 | 4.9500 | 0.69785 | 0.28490 | 4.2176 | 5.6824 | | |
| | APF | 6 | 0.0000 | 0.00000 | 0.00000 | 0.0000 | 0.0000 | | |
| **ZnL** | CPP-ACP | 6 | 0.0000 | 0.00000 | 0.00000 | 0.0000 | 0.0000 | 213.757 | **0.000*** |
| | BCD | 6 | 2.8667 | 0.48028 | 0.19607 | 2.3626 | 3.3707 | | |
| | APF | 6 | 0.0000 | 0.00000 | 0.00000 | 0.0000 | 0.0000 | | |
| **ClK** | CPP-ACP | 6 | 0.0000 | 0.00000 | 0.00000 | 0.0000 | 0.0000 | 4.868 | **0.023*** |
| | BCD | 6 | 0.3500 | 0.38859 | 0.15864 | -0.0578 | 0.7578 | | |

*Statistically significant

| Element | Group | N | Mean | SD | SE | 95% CI Lower | 95% CI Upper | F | p-value |
|---|---|---|---|---|---|---|---|---|---|
| | APF | 6 | 0.0000 | 0.00000 | 0.00000 | 0.0000 | 0.0000 | | |
| **MgK** | CPP-ACP | 6 | 0.0000 | 0.00000 | 0.00000 | 0.0000 | 0.0000 | 1.960 | **0.175** *(not significant)* |
| | BCD | 6 | 0.0000 | 0.00000 | 0.00000 | 0.0000 | 0.0000 | | |
| | APF | 6 | 0.1167 | 0.20412 | 0.08333 | -0.0975 | 0.3309 | | |
| *Statistically significant | | | | | | | | | |

In enamel specimens (Table 1), calcium levels varied significantly among the experimental groups. The APF-treated enamel specimens exhibited the highest mean calcium concentration (35.28 ± 1.08 wt%), followed closely by the BCD group (34.80 ± 0.54 wt%), whereas the CPP-ACP group demonstrated comparatively lower calcium values (24.50 ± 1.18 wt%). One-way ANOVA revealed that differences in calcium concentration among groups were statistically significant ($F = 234.019$, $p < 0.001$). The 95% confidence intervals further indicated minimal overlap between CPP-ACP and the other two treatment groups, suggesting comparatively reduced mineral deposition in the CPP-ACP group. A similar pattern was observed for phosphorus levels in enamel. The CPP-ACP group demonstrated the lowest phosphorus values (3.28 ± 0.40 wt%), whereas the BCD (13.17 ± 0.46 wt%) and APF (13.63 ± 0.46 wt%) groups exhibited markedly higher phosphorus concentrations. Statistical analysis confirmed significant differences among the groups ($F = 1040.713$, $p < 0.001$). The comparable phosphorus levels observed in the BCD and APF groups indicate that both agents facilitated enhanced calcium–phosphate deposition within the enamel substrate.

Evaluation of dentin specimens (Table 2) revealed notable differences in mineral preservation following treatment. In the CPP-ACP and BCD groups, calcium levels remained relatively stable, suggesting maintenance of mineral content and potential remineralisation effects within the dentinal structure. In contrast, the APF-treated dentin specimens showed lower calcium levels and increased carbon content, indicating reduced mineral deposition within dentin under the experimental conditions. Across the analysed elements, significant differences were observed among the treatment groups in both enamel and dentin, except for MgK in dentin, where no statistically significant difference was observed. In enamel specimens, the BCD and APF groups consistently showed higher concentrations of key remineralising elements, such as calcium, oxygen, and phosphorus, than the CPP-ACP group. The CPP-ACP specimens, however, showed relatively higher carbon levels, which may reflect greater organic residue associated with the paste formulation.

In dentin specimens, BCD demonstrated the most consistent elemental profile, maintaining calcium levels while facilitating increased phosphorus incorporation. Trace elements, including zinc, chlorine, and magnesium, were predominantly detected in the BCD group, suggesting material-specific mineral deposition characteristics. These observations collectively indicate that while APF and BCD promoted effective mineral deposition in enamel, BCD and CPP-ACP showed comparatively better mineral preservation within dentin substrates.

The morphological observations obtained from Field Emission Scanning Electron Microscopy (FESEM) shown in Fig. 1 & Fig. 2 supported the elemental findings. Demineralised control specimens exhibited pronounced surface irregularities with exposed enamel prisms and open dentinal tubules (Fig. 1a and 2a). In the CPP-ACP

and APF groups, FESEM images revealed scattered crystalline deposits on the enamel and dentin surfaces (Fig. 1b,c and 2b,c, respectively). In contrast, specimens treated with BCD demonstrated dense mineral deposition on enamel surfaces with obscured prism boundaries, indicating substantial remineralisation (Fig. 1d). In dentin, near-complete occlusion of dentinal tubules was observed in the BCD group, suggesting effective mineral integration and surface sealing (Fig. 2d). Overall, the combined FESEM and EDS findings indicate that the BCD solution demonstrated the most consistent remineralising behaviour across both enamel and dentin substrates, whereas APF showed stronger remineralising potential in enamel, and CPP-ACP demonstrated comparatively moderate mineral deposition under the present experimental conditions.

## Discussion

Remineralisation is a dynamic process in which calcium and phosphate ions are redeposited into partially demineralised dental tissues, thereby restoring mineral integrity and improving resistance to further acid attack. Casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) has been extensively investigated as a remineralising agent because it stabilises calcium and phosphate ions in an amorphous form and maintains a supersaturated environment that facilitates mineral deposition into enamel subsurface lesions. This mechanism contributes to the restoration of mineral balance and arrest of early carious lesions [1, 2].

The results of the present study demonstrated clear differences in the remineralisation patterns among the tested agents. Both FESEM and EDS analyses revealed that the BCD and APF groups showed greater calcium and phosphorus deposition in enamel than the CPP-ACP group. In contrast, dentin specimens showed comparatively better mineral preservation in the BCD and CPP-ACP groups, whereas APF demonstrated limited mineral integration within dentinal substrates. These findings suggest that the remineralisation efficacy of the tested agents may vary depending on the material composition and the structural characteristics of the dental tissue. Enamel, being highly mineralised, may respond more effectively to fluoride-based remineralisation, whereas dentin, with its tubular architecture and higher organic content, may benefit more from bioactive materials capable of deeper mineral integration. Artificial demineralisation of enamel specimens in the present study resulted in evident structural alterations characterized by surface irregularities and loss of prism definition, whereas dentin specimens exhibited open dentinal tubules without evidence of mineral deposition. These findings are consistent with previously reported experimental models investigating structural changes associated with mineral loss in dental tissues [7]. Due to the comparatively lower inorganic content of dentin relative to enamel, EDS analysis also demonstrated reduced mineral density in dentin specimens.

Among the tested remineralising agents, APF gel demonstrated a pronounced remineralising effect on enamel surfaces. FESEM analysis revealed scattered crystalline precipitates on the enamel surface, while EDS results confirmed significantly elevated calcium and phosphorus levels compared with CPP-ACP. Fluoride-based agents promote remineralisation primarily by forming fluorapatite crystals, which enhance enamel resistance to acid dissolution and facilitate mineral deposition [3, 4]. These observations are consistent with previous investigations reporting enhanced enamel microhardness following APF application [11]. Similarly, another study reported that application of APF gel using dielectrophoresis significantly increased microhardness of demineralised bovine enamel compared with CPP-ACP-treated specimens [13]. The strong enamel response observed in the present study, therefore, supports the well-established efficacy of fluoride-based therapies. CPP-ACP demonstrated comparatively lower calcium and phosphorus values in enamel compared with both BCD and APF groups. Although CPP-ACP contains bioavailable calcium and phosphate ions, its

remineralisation mechanism relies on stabilization of these ions and their gradual release over time. Consequently, the comparatively lower mineral deposition observed in this study may be attributed to the short experimental duration and the dependence of CPP-ACP on prolonged interaction with saliva and plaque biofilm for optimal remineralisation [1, 2].

In dentin specimens, CPP-ACP maintained relatively stable calcium levels, suggesting a protective or compensatory remineralising effect. However, the overall mineral gain was less pronounced than in the BCD group. These findings suggest that although CPP-ACP is an established remineralising agent, its short-term remineralisation potential may be comparatively moderate under controlled experimental conditions [14, 15]. The Bioactive Caries Detecting dye demonstrated the most consistent remineralising effect across both enamel and dentin substrates. FESEM analysis revealed dense deposition of apatite-like particles on enamel surfaces with obscured prism boundaries, indicating substantial mineral coverage [16]. In dentin specimens, near-complete occlusion of dentinal tubules was observed following treatment with BCD, suggesting effective mineral integration within the dentinal structure.

EDS analysis further showed that calcium and phosphorus levels in enamel treated with BCD were comparable to those in the APF group and significantly higher than those in the CPP-ACP group. The presence of nano-hydroxyapatite in the BCD formulation likely contributes to this enhanced remineralisation potential [7, 9]. Nano-hydroxyapatite has been reported to provide a direct source of bioavailable calcium and phosphate ions capable of integrating into the demineralised enamel lattice and facilitating mineral deposition [7, 9]. In dentin, BCD demonstrated superior mineral preservation when compared with both CPP-ACP and APF groups. Calcium levels remained stable and phosphorus incorporation was relatively higher, indicating improved mineral integration within the dentinal matrix. The presence of chitosan within the BCD formulation may further enhance remineralisation by improving adhesion and facilitating interaction between nano-hydroxyapatite particles and the dental substrate. Chitosan has been reported to possess chelating and adhesive properties that promote attachment of remineralising particles to enamel and dentinal surfaces [7, 8, 9, 17]. The scattered apatite-like particles observed in FESEM images support this mechanism and suggest that the combined action of nano-hydroxyapatite and chitosan contributes to enhanced dentinal tubule occlusion and improved substrate preparation for restorative bonding.

A major strength of the present study is the combined use of morphological and elemental analytical techniques to evaluate remineralisation. FESEM enabled high-resolution visualization of surface structural changes and mineral deposition patterns, whereas EDS allowed quantitative assessment of elemental composition, particularly calcium and phosphorus, which are critical indicators of remineralisation [9]. Furthermore, evaluating both enamel and dentin substrates enabled a comprehensive comparison of tissue-specific responses to the tested remineralising agents. However, certain limitations of this study should be considered. As an in vitro investigation, the experimental conditions could not completely replicate the complex dynamics of the oral environment, including salivary flow, pH fluctuations, dietary acids, and microbial biofilm activity. In addition, the duration of the study was relatively short and long-term effects of repeated application of the tested agents were not evaluated. Future investigations incorporating additional analytical techniques such as microhardness testing, transverse microradiography, or Raman spectroscopy may further validate the remineralisation outcomes. Furthermore, as BCD dye represents a relatively novel formulation, additional in vivo and clinical studies are required to evaluate its long-term clinical effectiveness and biocompatibility. Overall, the combined morphological and elemental analyses suggest that the novel bioactive caries detecting dye

demonstrates a broader remineralisation potential across both enamel and dentin substrates when compared with conventional remineralising agents.

## Conclusion

The Bioactive Caries Detecting (BCD) dye demonstrated promising remineralising potential on both enamel and dentin substrates. FESEM observations revealed dense mineral deposition on enamel surfaces and effective occlusion of dentinal tubules, while EDS analysis confirmed higher calcium and phosphorus levels comparable to those observed in the BCD treated group. Although Acidulated Phosphate Fluoride showed strong remineralisation in enamel, its effect in dentin was comparatively limited. CPP-ACP exhibited moderate remineralising capability, suggesting a gradual mineral deposition mechanism under the experimental conditions. The presence of nano-hydroxyapatite and chitosan in the BCD formulation may enhance mineral integration and dentinal tubule occlusion. Therefore, the bioactive dye may serve as a promising adjunct to minimally invasive caries management, combining diagnostic capability with therapeutic remineralisation.

# Clinical Significance

The findings of this study suggest that Bioactive Caries Detecting (BCD) dye may provide a dual clinical advantage by combining caries detection with remineralising potential. The dense mineral deposition on enamel and effective dentinal tubule occlusion observed in this study indicate that the material may help reduce dentin hypersensitivity and improve the quality of the bonding substrate during restorative procedures. Compared with conventional remineralising agents such as CPP-ACP and Acidulated Phosphate Fluoride, the bioactive formulation demonstrated consistent mineral preservation in both enamel and dentin.

## Future Research Directions

Although the present investigation demonstrated promising remineralisation potential of the bioactive caries detecting dye, further research is necessary to validate its clinical applicability. Future studies should evaluate the long-term remineralisation effects of the material under conditions that more closely simulate the oral environment, including salivary dynamics, pH fluctuations, and microbial biofilm activity.

## Abbreviations

• APF
Acidulated Phosphate Fluoride
• BCD
Bioactive Caries Detecting dye
• CPP-ACP
Casein Phosphopeptide–Amorphous Calcium Phosphate
• EDS
Energy Dispersive X-ray Spectroscopy
• FESEM
Field Emission Scanning Electron Microscopy

• NaF
Sodium Fluoride
• wt%
Weight percentage

## Declarations

**Ethics approval and consent to participate:**

Ethical approval for this study was obtained from the Institutional Ethics Committee of Institute of Dental Sciences, Siksha ‘O’ Anusandhan (Deemed to be University), Bhubaneswar, Odisha (Ref. No: IEC-IDS/IDS/SOA/2024/I-03). The study was conducted in accordance with the ethical standards of the institutional research committee and the principles of the Declaration of Helsinki. Extracted teeth used in the study were obtained from patients undergoing orthodontic extractions with prior informed consent for use in research.

**Consent for publication:**

Not applicable.

# Competing interests:

The authors declare that they have no competing interests.

# Funding:

The authors declare that no external funding was received for this study.

# Author Contribution

S.G., S.P. and M.M. conceptualised and designed the study.S.G., M.M., and S.P. were involved in the clinical procedures and data collection.S.G., M.M., and S.P. performed the statistical analysis and interpreted the data.S.G., M.M., S.P., and S.K. contributed to drafting and revising the manuscript critically for important intellectual content.S.G. and S.K. finalized the manuscript for submission.All authors reviewed and approved the final version of the manuscript.

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# Data Availability

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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## Figures

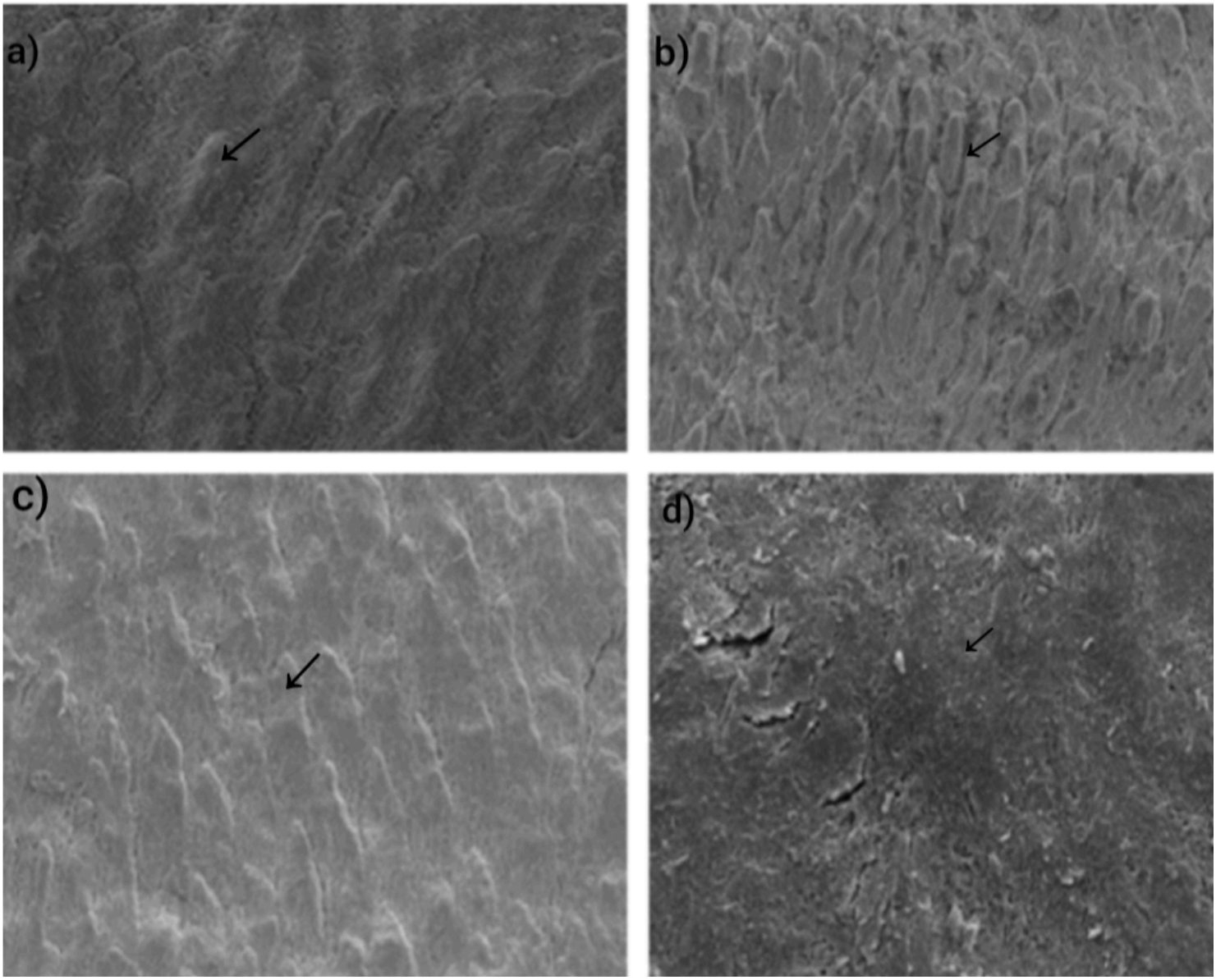


**Figure 1**

FESEM images of Enamels showing mineral deposition among the groups. a) Demineralised enamel, b) CPP-ACP group, c) APF group, d) BCD group.

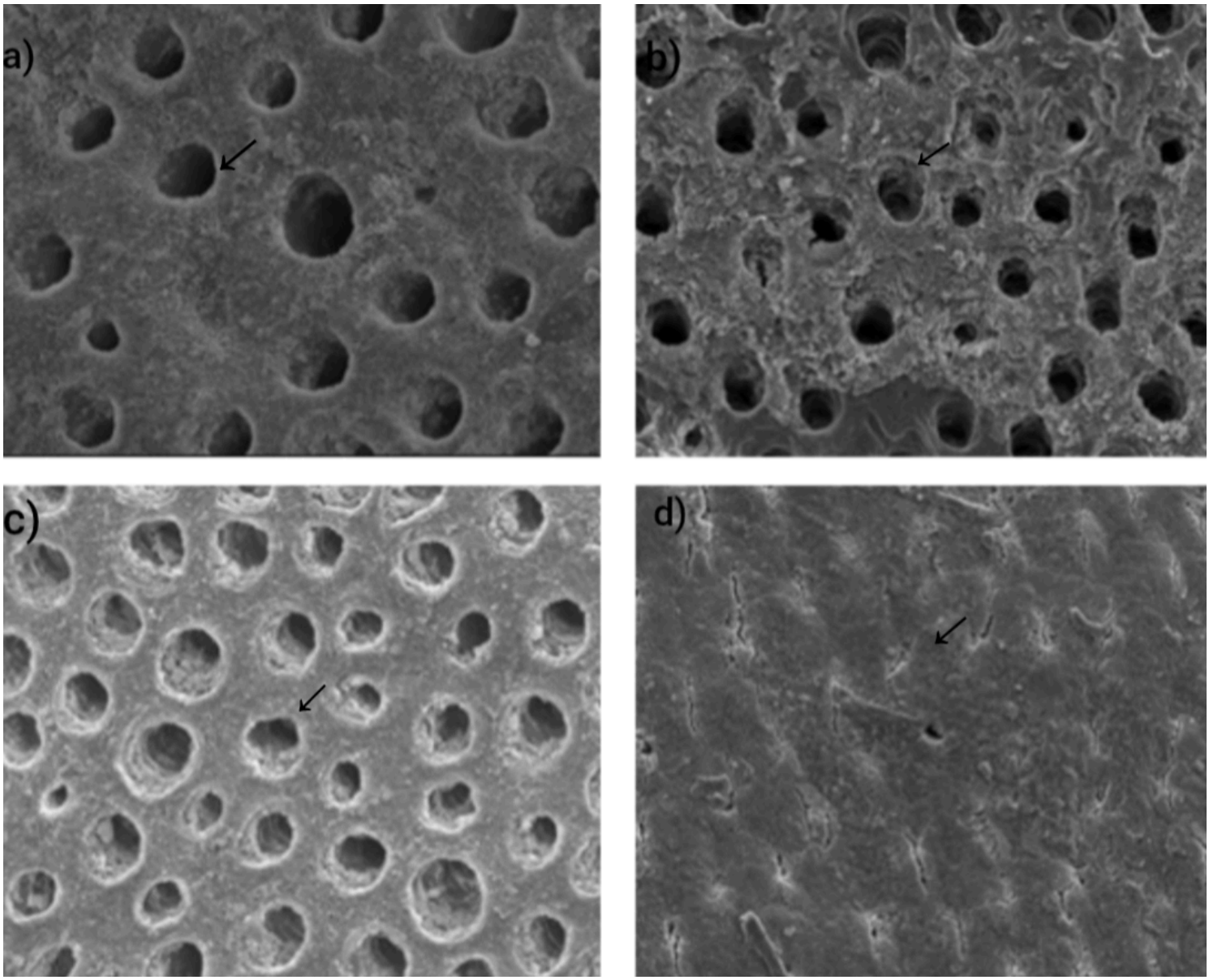



**Figure 2**

FESEM images of dentin showing tubule occlusion by mineral deposition among the groups: a) Demineralised dentin, b) CPP-ACP group, c) APF group, d) BCD group.