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Comparison of Phosphate Content in Hypomineralized and Sound Dental Enamel Using Raman Spectroscopy

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Abstract: **Introduction:** Molar hypomineralization is associated with alterations in the mineral composition of dental enamel, which can be evaluated using Raman spectroscopy. **Objective:** To compare phosphate concentration between healthy enamel and hypomineralized enamel using Raman spectroscopy as an analytical method. **Methods:** Sixteen extracted teeth with lesions consistent with molar hypomineralization were analyzed, selected according to inclusion and exclusion criteria. In each sample, healthy and hypomineralized areas were identified, and Raman spectra were obtained to determine the intensity of the peak corresponding to the phosphate ion. **Results:** The spectral intensity of the phosphate ion was consistently lower in hypomineralized areas compared to healthy ones. Analysis of variance (ANOVA) revealed statistically significant differences among clinical groups based on lesion color ($p < 0.05$). **Conclusions:** A significant decrease in phosphate concentration was observed in hypomineralized enamel, especially in lesions with greater clinical severity, which may be related to their increased fragility and susceptibility to fracture and caries progression.

Key words: hypomineralization, Raman spectroscopy, hydroxyapatite, dental enamel.

Comparación del contenido de fosfato en esmalte dental hipomineralizado y sano mediante espectroscopía Raman

Resumen: **Introducción:** La hipomineralización molar se asocia con alteraciones en la composición mineral del esmalte dental, las cuales pueden ser evaluadas mediante espectroscopía Raman. **Objetivo:** Comparar la concentración de fosfatos entre esmalte sano y esmalte con hipomineralización, utilizando espectroscopía Raman como método de análisis. **Métodos:** Se analizaron 16 muestras dentales extraídas con lesiones compatibles con hipomineralización molar, seleccionadas según criterios de inclusión y exclusión. En cada muestra se identificaron zonas sanas y zonas hipomineralizadas, y se obtuvieron espectros Raman para determinar la intensidad del pico correspondiente al ion fosfato. **Resultados:** La intensidad espectral del ion fosfato fue consistentemente menor en las zonas hipomineralizadas en comparación con las sanas. El análisis de varianza (ANOVA) reveló diferencias estadísticamente significativas entre los grupos clínicos según el color de la lesión ($p < 0,05$). **Conclusiones:** Se observó una disminución significativa en la concentración de fosfatos en el esmalte hipomineralizado, especialmente en las lesiones de mayor severidad clínica, lo que podría estar relacionado con su mayor fragilidad y susceptibilidad a la fractura y avance de lesiones de caries.

Palabras clave: hipomineralización, espectroscopía Raman, hidroxiapatita, esmalte dental.

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Comparação do conteúdo de fosfato em esmalte dentário hipomineralizado e saudável utilizando espectroscopia Raman

Resumo: **Introdução:** A hipomineralização molar está associada a alterações na composição mineral do esmalte dentário, as quais podem ser avaliadas por meio da espectroscopia Raman. **Objetivo:** Comparar a concentração de fosfatos entre esmalte saudável e esmalte com hipomineralização, utilizando a espectroscopia Raman como método de análise. **Métodos:** Foram analisadas 16 amostras dentárias extraídas com lesões compatíveis com hipomineralização molar, selecionadas de acordo com critérios de inclusão e exclusão. Em cada amostra, foram identificadas áreas saudáveis e áreas hipomineralizadas, e espectros Raman foram obtidos para determinar a intensidade do pico correspondente ao íon fosfato. **Resultados:** A intensidade espectral do íon fosfato foi consistentemente menor nas áreas hipomineralizadas em comparação com as saudáveis. A análise de variância (ANOVA) revelou diferenças estatisticamente significativas entre os grupos clínicos conforme a cor da lesão ($p < 0,05$). **Conclusões:** Observou-se uma diminuição significativa na concentração de fosfatos no esmalte hipomineralizado, especialmente nas lesões de maior gravidade clínica, o que pode estar relacionado à sua maior fragilidade e suscetibilidade à fratura e à cárie.

Palavras-chave: hipomineralização, espectroscopia Raman, hidroxiapatita, esmalte dentário.

Introduction

Molar–incisor hypomineralization (MIH), also referred to as molar hypomineralization (MH), is a qualitative defect of dental enamel characterized by well-demarcated opacities affecting primarily permanent molars and incisors, although other permanent and primary teeth may also be involved. The simultaneous involvement of both tooth types represents the most severe form of the disorder; however, the presence of opacities limited to incisors without the involvement of one or more first permanent molars is not considered true MH[1,2].

Currently, MH is described as a defect of systemic origin with multifactorial etiology, affecting one to four first permanent molars. This alteration is associated with changes in the arrangement of hydroxyapatite crystals, attributed to increased retention of proteins such as serum albumin and type I collagen, which interfere with the normal mineralization process during enamel maturation [3,4]. Clinically, affected enamel presents as white, cream, yellow, or brown opacities, and in severe cases, post-eruptive enamel breakdown may occur.

The clinical severity of MH is variable, ranging from mild opacities to complex cases with enamel fracture, hypersensitivity, atypical restorations, and accelerated caries development. These alterations significantly compromise the coronal structure[2,5]. At the histochemical level, enamel affected by MH exhibits up to 19% lower mineral density compared to sound enamel, with reduced calcium and phosphorus levels and up to 21 times higher protein content[6].

This defect has become increasingly prevalent in clinical practice, representing a particular challenge for pediatric dentists, as manifestations occur at early ages [2]. Its global prevalence ranges widely, from 2.4% to 40.2%, affecting both permanent and primary dentitions[7].

Recent research has identified up to five distinct phenotypes of hypomineralization, and a new term has been proposed: hypomineralization of other permanent teeth (HOPT), which includes premolars and may share etiology with MH and MIH[8,9]. Despite more than two decades of investigation, the exact etiology of MH remains unclear, reinforcing its recognition as a public health concern and justifying continued research into its risk factors, impact on quality of life, and clinical management[10].

In this context, Raman spectroscopy has emerged as a highly effective, non-destructive technique for the analysis of biological tissues, including dental enamel. This vibrational technique provides precise structural and chemical information, functioning as a molecular "fingerprint" for the identification of organic and inorganic compounds [11]. Several studies have employed Raman spectroscopy to investigate structural defects in enamel affected by MH, particularly focusing on the concentration of the phosphate ion (v1), a key component of hydroxyapatite[12–15].

Findings indicate that the inorganic phosphate content in teeth affected by MH is lower compared to sound enamel, influencing acid-etching response and the adhesion of restorative materials. This reduced mineralization is associated with increased protein content and disorganization of hydroxyapatite crystals, compromising the structural integrity of the tissue [16].

The present study analyzed phosphate concentration in sound dental enamel and in hypomineralized enamel with different clinical manifestations (white, cream, yellow, and brown) using Raman spectroscopy. The objective was to compare phosphate ion content between both conditions and to evaluate differences according to the clinical color of the lesions.

Materials and Methods

Study Design and Ethical Considerations

This in vitro experimental study was conducted at the Research Laboratory of the Postgraduate Program in Dentistry at the University of Costa Rica (UCR). Because the study used extracted teeth classified as anatomical pathological waste and contained no identifiable patient information, formal review by the Scientific Ethical Committee (CEC-UCR) was not required.

A total of 16 extracted human permanent teeth were collected from the dental clinics of the Faculty of Dentistry. All adult patients, parents, or legal guardians had previously signed informed consent forms authorizing the use of extracted teeth for educational or research purposes. Tooth extraction was unrelated to this investigation.

Inclusion Criteria

- Single- or multi-rooted permanent teeth with a clinical diagnosis of molar hypomineralization (MH).

- Coronal surfaces presenting well-demarcated white, cream, yellow, or brown opacities, or post-eruptive enamel breakdown.
- Permanent teeth with atypical restorations preserving hypomineralized areas.
- Lesions located in the middle third of the crown or on occlusal cusps.
- Presence of post-eruptive enamel fractures associated with opacities.
- Severely damaged teeth retaining identifiable hypomineralized areas.
- Teeth adequately preserved for spectroscopic analysis.

Exclusion Criteria

- Fractured teeth without evidence of hypomineralization.
- Atypical restorations not involving hypomineralized enamel.
- Cavitated carious lesions without hypomineralized enamel margins.
- Enamel defects associated with etiologies other than MH.

Sixteen teeth meeting the criteria were included. Six additional samples were excluded due to alternative diagnoses such as fluorosis, restorations without hypomineralized areas, or cavitated caries.

Samples were cleaned using a toothbrush, water, and an ultrasonic cavitron device. They were subsequently stored in glass containers with distilled water at 20 °C until analysis.

Each molar was classified according to lesion color into three categories based on the European Academy of Paediatric Dentistry (EAPD) criteria:

- White/cream
- Yellow/brown
- Post-eruptive enamel breakdown associated with MH

Spectroscopic Analysis

Raman Spectral Acquisition

Raman analysis was performed using a confocal Raman microscope (WiTec, model PRO-L7A2S-10; Figure 1), equipped with a 785 nm laser operating at 100 mW power. The measurement spot size was approximately 1–2 µm using a 40× objective lens. Each spectrum was acquired with an integration time of 0.5 seconds per scan.

Prior to measurement, the instrument was calibrated, and samples were dried to minimize water fluorescence interference (Rayleigh scattering). Each tooth was secured with wax on a sample holder.

Figure 1. *Confocal Raman microscope (WiTec, model PRO-L7A2S-10).*

Measurements were performed in both hypomineralized areas and visually sound enamel areas of the same tooth, which served as internal controls. Environmental conditions were maintained at 26 °C.

Data Preprocessing

Spectral data were visualized and processed using Spectragryph® software (version 1.2.16, Dr. Friedrich Menges), which allowed extraction of energy values (x-axis), intensity values (y-axis), and peak area measurements.

Preprocessing steps included baseline correction, smoothing, and outlier removal (Figure 2).

The phosphate v1 (PO_4^{3-}) peak, corresponding to the inorganic mineral component (hydroxyapatite), was identified primarily in the spectral region between 958 and 960 cm^{-1}. Enamel crystallinity was assessed by calculating the 960/1070 cm^{-1} ratio.

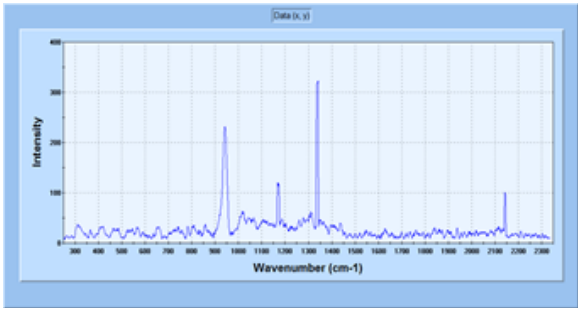

Figure 2. *Raman spectrum obtained from a representative sample.*

Statistical Analysis

Data were tabulated in Microsoft Excel and analyzed using SPSS software (version 28.0).

Normality was assessed using the Shapiro–Wilk test prior to inferential analysis.

- Paired Student's t-test was used to compare phosphate values between sound and hypomineralized enamel.
- One-way analysis of variance (ANOVA) was used to compare phosphate concentrations according to clinical lesion color.

Statistical significance was set at $\alpha = 0.05$. When normality assumptions were not met, non-parametric analyses were considered.

Results

A total of 16 permanent dental samples meeting the inclusion criteria were analyzed. Six additional teeth were excluded due to fluorosis, cavitated caries, or restorations without hypomineralized areas. Evaluation was performed using Raman spectroscopy, focusing on the spectral intensity of the phosphate ion v1 (PO_4^{3-}) peak as an indicator of enamel mineral content.

Comparison Between Sound and Hypomineralized Enamel

Each tooth was evaluated in two regions: a clinically and visually sound area (control enamel) and an area exhibiting hypomineralization. The mean spectral intensity of the phosphate ion for each sample is presented in Table 1.

Overall, the mean spectral intensity of the phosphate ion in sound enamel was 113.98 cm^{-1}, whereas in hypomineralized areas it decreased to 44.44 cm^{-1}, representing an average reduction of 61% in the mineral-associated signal.

Table 1. *Mean spectral intensity (cm^{-1}) of the phosphate ion in sound and hypomineralized enamel.*

Sample	Sound Enamel	Hypomineralized Enamel
1	78,42	64,34
2	42,14	33,40
4	52,73	41,85
5	242,30	39,07
6	103,00	85,43
7	61,22	22,22
8	147,80	59,26
9	70,41	69,32
11	98,68	33,59
12	412,10	79,27
13	87,82	18,83
15	50,09	46,45
16	58,71	24,43
20	230,60	34,09
21	35,83	26,03
22	51,87	33,56

Fuente: Elaboración propia a partir de los datos obtenidos por espectroscopía Raman.

This reduction is consistent with the mineral loss clinically observed in hypomineralized lesions and is clearly illustrated in Figure 3, where most samples demonstrate a marked decrease in phosphate spectral intensity in affected areas.

To assess statistical significance, a paired Student's t-test was performed, yielding a mean difference of 69.53 cm^{-1} ($p = 0.10$). Although this value did not reach conventional statistical significance ($\alpha = 0.05$), the magnitude of the reduction suggests a clinically relevant trend. These findings highlight the need for studies with larger sample sizes to further validate the observed differences and explore their diagnostic and therapeutic implications.

Comparison According to Clinical Color of Hypomineralized Enamel

Hypomineralized enamel areas were classified into three clinical categories based on visual appearance: white–cream,

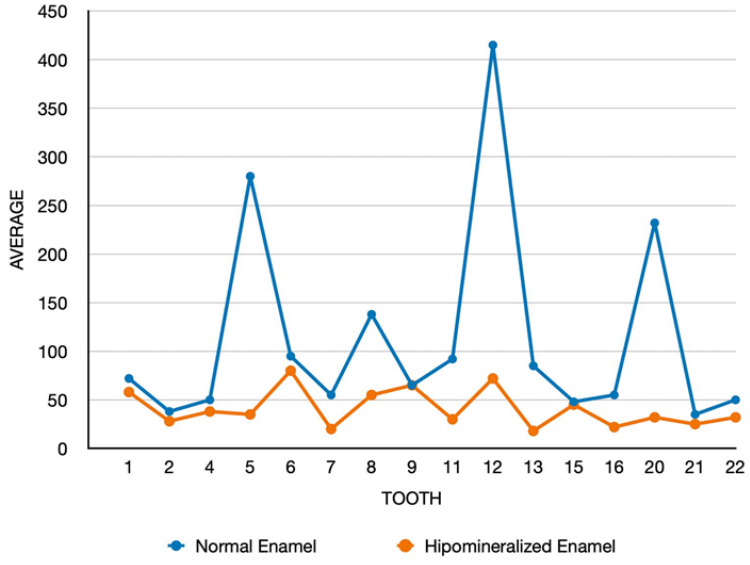

Figure 3. *Mean spectral intensity of phosphates in sound and hypomineralized enamel by sample.*

yellow–brown, and brown with structural loss. For each group, the mean percentage reduction in phosphate v1 spectral intensity was calculated relative to the corresponding sound enamel areas (Table 2).

These results suggest a direct relationship between clinical severity and the magnitude of mineral loss, with greater reductions observed in darker or structurally compromised lesions. This trend is illustrated in Figure 4, where the percentage reduction increases with increasing clinical severity.

To determine whether these differences were statistically significant, a one-way ANOVA was performed (Table 3).

ANOVA revealed statistically significant differences among clinical categories only in hypomineralized enamel (p = 0.037), whereas no significant differences were observed in sound enamel (p = 0.933).

Table 2. *Mean percentage reduction of phosphate content according to clinical color.*

Clinical Group	Mean Reduction (%)
Brown with structural loss	85%
Yellow–brown	81%
White–cream	66%

Pairwise Comparisons Among Clinical Groups

To further explore the relationship between clinical color and mineral content, pairwise comparisons were performed among the three clinical groups (white–cream, yellow–brown, and structural loss). Spectral intensities were compared for both sound and hypomineralized areas.

The results demonstrate that in hypomineralized areas, phosphate spectral

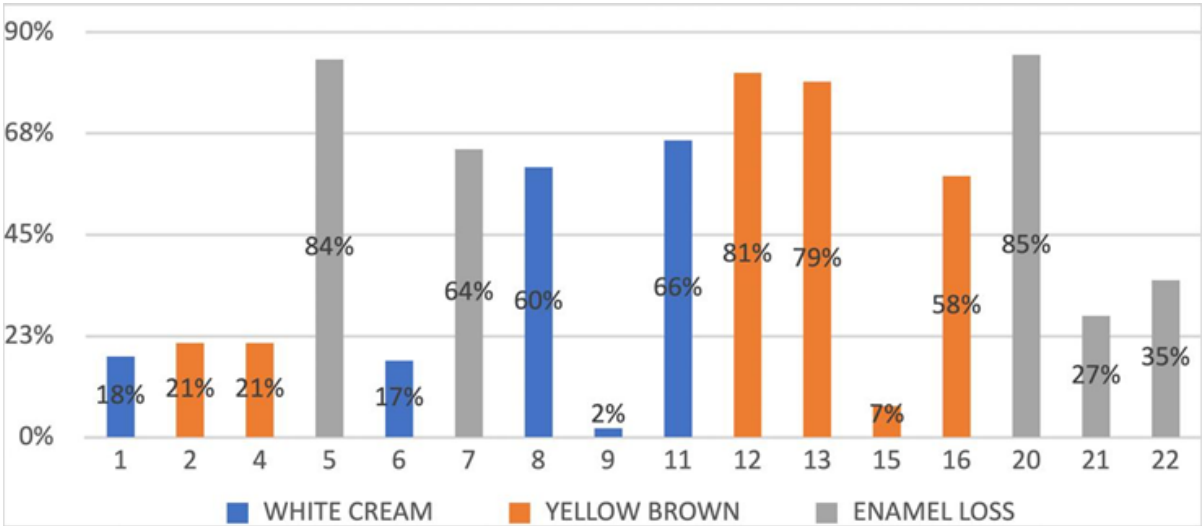

Figure 4. *Percentage reduction of phosphate content according to clinical color of hypomineralized enamel*

Table 3. *ANOVA results for spectral intensity according to clinical color groups*

Group	Sum of Squares	df	Mean Square	F	*p-value*
Sound enamel	1628.91	2	814.46	0.07	0.933
Hypomineralized enamel	2598.33	2	1299.16	4.31	0.037*

*$p < 0.05$ indicates statistical significance.

intensity decreases progressively with increasing clinical severity. White–cream enamel showed intermediate phosphate levels, yellow–brown lesions exhibited greater reductions, and lesions with structural loss presented the lowest recorded values. This gradient remained evident when compared with their corresponding sound enamel areas.

Table 4. *White–cream vs. yellow–brown enamel*

Group	Mean	SD	N
Sound – White–cream	99.66	30.15	5
Sound – Yellow–brown	117.27	145.29	6
Hypomineralized – White–cream	62.39	18.65	6
Hypomineralized – Yellow–brown	40.71	21.53	6

Table 5. *White–cream vs. structural loss*

Group	Mean	SD	N
Sound – White–cream	94.63	30.15	5
Sound – Structural loss	124.36	102.8	5
Hypomineralized – White–cream	62.39	18.65	5
Hypomineralized – Structural loss	30.99	6.76	5

Table 6. *Yellow–brown vs. structural loss.*

Group	Mean	SD	N
Sound – Yellow–brown	117.27	145.29	6
Sound – Structural loss	124.36	102.8	5
Hypomineralized – Yellow–brown	40.71	21.53	5
Hypomineralized – Structural loss	30.99	6.76	5

Summary of Findings

The findings confirm that enamel mineral content, measured through phosphate v1 spectral intensity, is lower in hypomineralized areas compared to sound enamel. Although the overall reduction (61%) did not reach statistical significance (p = 0.10), its magnitude suggests potential clinical relevance, particularly in diagnostic assessment, preventive management, and restorative material selection.

Subgroup analysis revealed statistically significant differences in phosphate intensity according to clinical color (p = 0.037), indicating that observable lesion color may reflect the underlying degree of hypomineralization.

Pairwise comparisons further support this interpretation, demonstrating that brown lesions with structural loss were the most severely demineralized, followed by yellow–brown lesions, and finally white–cream lesions.

Discussion

The predominant mineral component of dental enamel is hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], whose inorganic phase is largely composed of phosphate ions. In the present study, Raman spectroscopy was used to quantify the spectral intensity of the phosphate v1 peak (approximately 960 cm^{-1}) as an indicator of enamel mineralization. The results demonstrated that phosphate content in hypomineralized enamel (MH) was markedly lower than in sound enamel, supporting previous findings regarding structural alterations in this tissue[16].

Overall, an average reduction of 61% in phosphate signal intensity was observed in hypomineralized areas, with more pronounced reductions in clinically severe lesions, particularly those classified as brown with structural loss. This trend is consistent with the findings of Fraser et al., who reported an association between clinical color severity and enamel involvement [15], as well as Cerdas et al., who documented greater structural compromise in brown lesions of permanent teeth affected by MH [17].

Previous studies have described MH enamel as more porous, with abnormal retention of proteins such as serum albumin and type I collagen, and irregular prism organization [18,19]. These microstructural alterations explain its reduced mechanical resistance and increased susceptibility to post-eruptive enamel breakdown. In the present study, brown lesions with structural

loss exhibited the lowest phosphate concentrations, followed by yellow–brown and white–cream lesions. This gradation was statistically significant in the ANOVA analysis ($p = 0.037$), supporting the hypothesis that clinical color may serve as an indicator of enamel structural quality.

From a clinical perspective, the reduction in phosphate content has important implications. Lower mineralization and increased porosity negatively affect the response of MH enamel to acid etching and compromise the adhesion of restorative materials [20,21]. Recent studies have reported lower clinical success rates for adhesive restorations placed on hypomineralized enamel, particularly when optimized adhesive systems or specific infiltration techniques are not employed [23–25].

The present study contributes to the understanding of the chemical composition of MH enamel using a non-destructive technique such as Raman spectroscopy. This method allows tissue analysis without structural alteration, as also emphasized by González-Solís et al. and Zepeda-Zepeda et al., who validated its utility in diagnosing enamel defects such as fluorosis [26,27]. Additionally, Raman spectroscopy has proven effective in analyzing the chemical structure at the dentin–enamel junction and characterizing enamel crystallization patterns [28].

The reduced phosphate content observed in MH enamel has been associated with increased retention of proteins, including carbonates and albumin, which has been suggested as a factor facilitating bacterial colonization and caries progression [18,19,29]. This reinforces the importance of early identification of MH lesions to implement more effective preventive protocols, particularly in children at elevated risk.

The spectral analysis conducted in this study confirmed, in agreement with existing literature, that the phosphate v1 peak around 960 cm^{-1} correlates directly with hydroxyapatite crystal concentration [14]. This relationship allows differentiation between sound and affected enamel and even discrimination among different degrees of clinical severity based on lesion color, representing a potential pathway for more precise and minimally invasive diagnostic approaches.

Currently, there is limited literature in Latin America comprehensively documenting the mineral composition of MH enamel and its clinical implications. Studies such as that of Natarajan et al. (2015) [30] demonstrate the potential of this research field; however, further investigations are needed to explore these characteristics in different populations and clinical contexts.

This study supports the clinical utility of Raman spectroscopy as a tool for characterizing enamel hypomineralization. The findings encourage future research with larger sample sizes to validate the relationship between clinical lesion appearance, chemical composition, and restorative performance. A deeper understanding of the structural limitations of hypomineralized enamel may foster the development of materials and techniques specifically adapted to these defects, ultimately improving the quality of life of affected patients.

It is important to acknowledge the methodological limitations of this study, particularly the sample size (n = 16), which may have contributed to the overall difference in phosphate concentration (61% reduction) not reaching statistical significance. As an in vitro investigation, the results reflect the static chemical composition of extracted enamel and do not account for the dynamic complexity of the oral environment, including salivary influence or in vivo lesion progression. Future studies should include larger samples and complement chemical analysis with direct mechanical testing (e.g., microhardness) to quantitatively correlate phosphate reduction with clinically inferred enamel fragility.

Conclusions

This study demonstrated, through Raman spectroscopy, a marked reduction in phosphate concentration in hypomineralized enamel compared with sound enamel. The decrease was more pronounced in clinically severe lesions, particularly those classified as brown with structural loss, whereas white–cream lesions exhibited less pronounced mineral alterations. These findings suggest that clinical color may serve as an indicator of the degree of demineralization and, consequently, enamel fragility.

Raman spectroscopy proved to be a useful, non-invasive, and precise technique for the chemical analysis of dental enamel, offering promising applications in both clinical practice and research settings. Finally, the results underscore the importance of developing restorative protocols specifically tailored to hypomineralized enamel, taking into account its structural particularities and distinct clinical behavior.

Conflict of Interest and Funding

The authors declare no conflicts of interest and no external funding related to the results presented in this study.

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