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***Abstract*—Endodontic irrigation protocols based on sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) are essential for effective root canal disinfection, nevertheless they can induce chemical alterations of coronal dentin that compromise the stability of subsequent adhesive restorations. This study investigates the effect of a clinically relevant irrigation protocol (5% NaOCl followed by EDTA for 30 min) on dentin chemical composition, comparing specimens subjected to direct irrigation with specimens protected by a universal adhesive applied according to the Pre-Endodontic Dentin Sealing (PEDS) protocol. Two experimental groups were compared: Group A, subjected to direct irrigation with 5% NaOCl followed by 30 min of EDTA, and Group B, which received PEDS treatment before the same irrigation protocol. Raman spectroscopy was performed directly on dentin surfaces before and after irrigation, acquiring multiple spectra on the cavity. Mineral- and collagen-related Raman bands were analyzed, and quantitative intensity ratios were calculated to assess demineralization and organic matrix degradation. Raman analysis revealed a significant reduction in phosphate-related signals and mineral-to-matrix ratios after irrigation in non-protected specimens, with marked changes in amide-related ratios indicative of collagen degradation and conformational disorder. In contrast, specimens treated with PEDS exhibited minimal variations in both mineral and organic Raman parameters, demonstrating effective preservation of dentin chemical integrity. These findings indicate that NaOCl and EDTA induce measurable chemical modifications of dentin that may negatively affect adhesive performance, while Pre-Endodontic Dentin Sealing significantly mitigates irrigant-induced damage. Raman spectroscopy proved to be a sensitive and non-destructive tool for characterizing dentin–irrigant–adhesive interactions and supporting optimized protocols for durable post-endodontic restorations.**

***Index Terms*—Raman spectroscopy, Material Characterization, Restorative Dentistry, Dentin Treatment, Endodontic, Irrigants.**

I. Introduction

The effective management of endodontic irrigants is a critical factor for preserving the chemical and structural integrity of dentin during root canal therapy, while minimizing adverse effects on subsequent adhesive restorative procedures. Sodium hypochlorite (NaOCl) remains the most widely used endodontic irrigant due to its strong antimicrobial and organic tissue–dissolving properties, whereas ethylenediaminetetraacetic acid (EDTA) plays a complementary role by chelating calcium ions and removing the inorganic component of the smear layer. Despite their clinical effectiveness in cleaning debris, the sequential application of NaOCl and EDTA may induce significant alterations in both the organic (collagen) and inorganic (apatite) phases of dentin, with effects strongly dependent on concentration, exposure time, and irrigation sequence [1], [2].

Previous studies have shown that NaOCl degrades dentinal collagen in a concentration- and time-dependent manner, leading to an increased apatite-to-collagen ratio and reduction in dentin mechanical and adhesive properties. Although EDTA is essential for smear layer removal, prolonged or aggressive application may worsen dentin demineralization and erosion, particularly when used after NaOCl [3], [4]. These chemical modifications are clinically relevant because dentin represents the primary substrate for adhesive restorations, and changes in its composition can directly compromise the formation and long-term stability of the hybrid layer.

The hybrid layer is a complex interfacial structure formed by infiltration of resin monomers into demineralized dentin and exposed collagen fibrils. Although modern universal ("all-in-one") adhesives have simplified clinical protocols and reduced technique sensitivity by combining etching, priming, and bonding in a single step, the dentin-adhesive interface

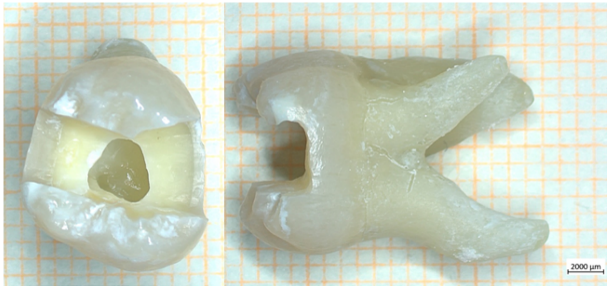

Fig. 1: Tooth sample with 2 mm cavity depth from the coronal margin

remains the weakest link in composite restorations [5], [6]. Despite these advances, the hybrid layer remains susceptible to degradation phenomena such as nanoleakage, hydrolytic breakdown, and enzymatic collagen degradation, especially when the underlying dentin substrate has been chemically altered by endodontic irrigants. In this context, the application of a universal adhesive before the endodontic irrigation, known as *Pre-Endodontic Dentin Sealing* (PEDS), has been proposed as a strategy to protect freshly exposed dentin from the chemical effects of irrigants and to improve the quality and durability of the adhesive interface [7]–[9]. Scotchbond Universal has been shown to form a thin protective barrier capable of limiting irrigant-induced degradation while maintaining stable bond strength independently of the irrigant used, although the chemical condition of the underlying dentin substrate remains a key factor influencing adhesive performance [10], [11].

Raman spectroscopy represents a powerful, non-destructive analytical technique for the in situ chemical characterization of dentin and adhesive interfaces, providing quantitative information on mineral and organic components with high spatial resolution and without the need for sample manipulation [12], [13]. Changes in Raman spectral parameters, such as mineral-to-matrix ratios and collagen-related band intensities, offer objective markers of dentin demineralization, deproteinization, and collagen structural integrity following endodontic procedures [14]–[16].

Within this framework, the present study investigates the effect of different endodontic irrigation protocols based on 5% NaOCl followed by EDTA for 30 min. Two experimental conditions were tested: Group A, subjected to irrigation without adhesive protection, and Group B, treated with the same irrigation protocol after application of Scotchbond Universal according to the PEDS approach. Raman spectroscopy was used to assess changes in dentin composition and collagen structure due to irrigants, to provide measurement-based evidence to support optimized irrigation and adhesive strategies that preserve dentin integrity and enhance the long-term durability of post-endodontic restorations.

II. Materials and Methods

A. Specimen Preparation

Extracted human teeth free of caries, cracks, restorations, or structural defects were selected for this study. All specimens were visually inspected and clinically evaluated by an experienced dentist to confirm their suitability. After extraction, residual soft tissues were carefully removed, and the teeth were thoroughly cleaned. Samples were stored in distilled water at room temperature until testing in order to prevent dehydration and preserve dentin properties.

A cavity was performed on the mesial surface of the tooth using a conical bur to a depth of 2 mm from the coronal margin. The 2 mm depth was measured from the lowest and most mesial point achievable, using the occlusal plane as the reference. A total of ten teeth were randomly allocated to two experimental groups ($n = 5$ per group): Group A, representing unprotected dentin, and Group B, which received a protective adhesive layer before irrigation according to the PEDS procedure. The two experimental groups followed the same irrigation protocols. An example of a tooth used for analysis with a 2 mm cavity is shown in Fig. 1.

B. Pre-Endodontic Dentin Sealing (PEDS)

The samples of Group B underwent adhesive application immediately after cavity preparation and before irrigation. The dentin hybridization was performed with a Universal adhesive system (Scotch Bond Universal, 3M, USA). The application was carried out following the steps indicated by the manufacturer: use a 37% phosphoric acid on enamel and dentin surface, followed by a thorough water rinse step to remove it from the tooth surface. The bond was actively applied for 20 s; gently air-dried for 5 s for air-thinning. Light-cure was performed for 20 s using a MultiLED curing unit. Following polymerization, specimens were subjected to the same irrigation protocol as non-protected samples.

C. Irrigation Protocols

All specimens were subjected to a standardized endodontic irrigation protocol consisting of 5% NaOCl followed by EDTA for a total exposure time of 30 min. Based on the presence or absence of adhesive protection, samples were divided into two experimental groups:

- **Group A**: 5% NaOCl followed by EDTA for 30 min, without adhesive protection
- **Group B**: 5% NaOCl followed by EDTA for 30 min, with PEDS using Scotchbond Universal

All irrigation procedures were performed under controlled laboratory conditions. Upon completion of the irrigation protocol, specimens were thoroughly rinsed with distilled water to remove residual chemicals and gently air-dried prior to Raman spectroscopic analysis.

For each sample, Raman spectroscopy was used to evaluate the chemical alteration in dentin induced by the irrigation protocol. Raman measurements were performed both before (T0) and after irrigation (T1). Five spectra were collected from different points on the exposed dentin surface of the cavity to account for the local variability.

All spectra were acquired using the same experimental parameters. Quantitative Raman metrics related to the mineral and organic components of dentin were extracted and used for comparative analysis. The experimental design allowed the assessment of the chemical changes in dentin induced by irrigation and the evaluation of the protective effect of Pre-Endodontic Dentin Sealing (PEDS) by comparing protected and non-protected samples, as well as pre- and post-irrigation conditions within the same sample.

III. Raman Spectroscopic Measurements

Raman measurements were conducted directly on polished dentin surfaces using a modular, portable Raman spectroscopy system (BWTEK, USA), equipped with a 1064 nm monochromatic laser source and a BTC284N spectrometer. The spectrometer provided a spectral range from 100 cm^{-1} to 2500 cm^{-1} with a spectral resolution of 10.56 cm^{-1}. Signal acquisition was performed using a charge-coupled device (CCD) detector. A compact optical microscope module (BAC151), integrated into the Raman system, enabled accurate focusing and spatial localization of the laser beam on the dentin surface. All measurements were performed using 40% of the maximum laser output power in order to ensure an adequate signal-to-noise ratio while avoiding thermal damage to the biological tissue.

For each acquisition, an integration time of 40 s was used, with four accumulations per spectrum to improve spectral quality. Measurements were carried out using a 20× objective lens, corresponding to an effective sampling area of approximately 90 μm. Four repeated acquisitions were recorded at each measurement site to ensure repeatability.

To account for local heterogeneity of dentin, five Raman spectra were collected from different locations within each cavity for every experimental condition. Spectral data were subsequently averaged and processed for quantitative analysis of mineral and organic components. Subsequently, baseline correction to remove background signals was performed to observe the most prominent peaks and to assess the chemical composition of dentin, focusing on the integrity of hydroxyapatite and collagen. Typical Raman spectra of human dentin with the key peaks analyzed in this study are shown in Fig. 2, before (T0) and after experimental protocol (T1) with irrigants.

IV. Results

Raman spectroscopy was employed to investigate the chemical modifications of dentin induced by the irrigation protocols by analyzing characteristic vibrational bands of its mineral and organic components. Representative Raman spectra acquired before and after the irrigation protocol for the group without adhesive (Group A) are illustrated in Fig. 2. Raman-derived quantitative parameters are presented as boxplots to illustrate data distribution and variability for each analyzed metric. For each parameter, the box represents the interquartile range, encompassing 50% of the data between the 25th and 75th percentiles, with the central line indicating the median value. Whiskers extend to the minimum and maximum measured values, providing a visual representation of data dispersion within each experimental condition.

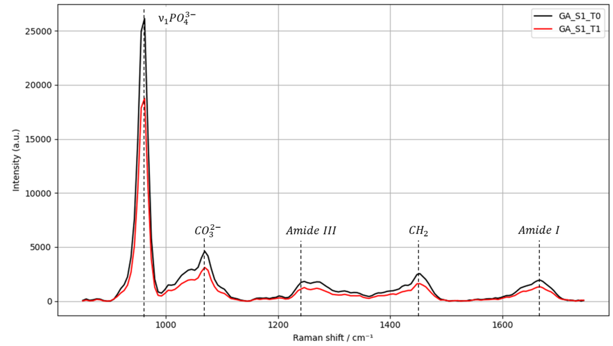

Fig. 2: Representative Raman spectra acquired from the sample without adhesive protection. The black spectrum (T0) corresponds to the sample before irrigation, while the red spectrum (T1) was collected after treatment with irrigants.

To quantify alteration in dentin, different specific bands were monitored: the symmetric stretching vibration of phosphate groups in hydroxyapatite, located at approximately 960 cm^{-1} (phosphate ν_1), served as a marker for mineral content, while the carbonate band at approximately 1070 cm^{-1} was analyzed to assess changes in mineral maturity and chemical stability [16], [17].

A reduction in the phosphate peak intensity (I_{960}) was observed in both experimental groups following sequential irrigation with NaOCl and EDTA, as shown in Fig. 3. This decrease serves as a direct spectral marker of hydroxyapatite dissolution driven by the Ca^{2+} chelation action of EDTA. This effect is further amplified by the preceding NaOCl-mediated deproteinization, which dissolves collagenous and non-collagenous proteins, thereby increasing the surface exposure of embedded hydroxyapatite crystals to the chelating agent. However, the intensity drop was more pronounced in Group A (unprotected dentin) compared to Group B (PEDS) due to the protective efficacy of the PEDS protocol as a chemical barrier, that mitigates the direct impact of the irrigants on the mineral phase.

Regarding the organic matrix, the integrity of the collagen network was evaluated through the Amide I band at approximately 1660 cm^{-1}, corresponding to $C = O$ stretching vibrations, and the Amide III band in the range of 1240–1270 cm^{-1}, which is sensitive to collagen secondary structure [18]. A significant increase in the Amide III/CH_2 ratio was observed after irrigation in Group A, whereas only a slight increase was detected in Group B, as shown in Fig. 4. This significant rise in the unprotected group indicates a relative loss of non-collagenous components and suggests a denatured matrix that is more susceptible to structural collapse and enzymatic degradation. In parallel, the Amide I/Amide III ratio remained nearly unchanged in the PEDS group while a slight decrease was detected in Group A, Fig. 5. This behavior is consistent with the oxidative action of NaOCl, which preferentially disrupts structured collagen by breaking peptide bonds, leading to a reduction in the Amide I contribution relative to Amide III. This biochemical shift highlights the

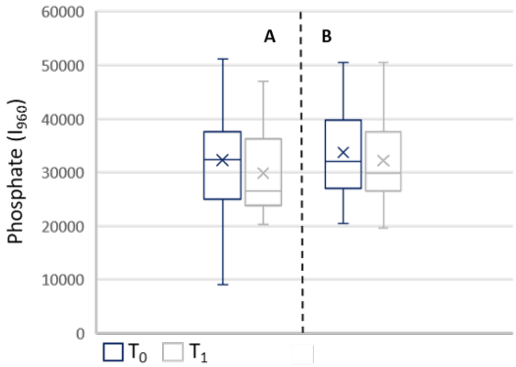

Fig. 3: Boxplot of phosphate Raman band intensity at $\sim$960 cm^{-1} for Group A (without adhesive) and Group B (with adhesive). Blue boxes represent measurements acquired before irrigation (T0), while gray boxes correspond to Raman measurements performed after the irrigation protocol (T1).

denaturation and solubilization of superficial collagen fibrils, leaving an organic matrix that is both impoverished and conformationally altered. Furthermore, the mineral-to-matrix ratio (PO_4 ν_1/CH_2) decreased slightly for Group A (indicates mild net mineral loss relative to the organic matrix after treatment), while remaining stable for Group B, as illustrated in Fig. 6. This result confirms that EDTA-induced chelation predominantly drives mineral loss in non-protected dentin, exposing collagen that has already been oxidatively modified by NaOCl. Such a condition is unfavorable for effective adhesive infiltration and is associated with reduced hybrid layer stability. Conversely, the stable mineral-to-matrix ratio observed in the PEDS group indicates that the adhesive layer effectively mitigated irrigation-induced demineralization. Overall, NaOCl treatment resulted in preferential degradation of structured collagen (Amide I reduction), enriching the residual organic matrix in less ordered components (Amide III/CH_2 increase), while EDTA amplified relative mineral loss (mineral-to-matrix decrease). The application of Pre-Endodontic Dentin Sealing effectively interrupted this degradation pathway, preserving both mineral and organic components and maintaining a dentin substrate more favorable for durable adhesive bonding.

V. Conclusion

This study evaluated the chemical effects of a clinically relevant endodontic irrigation protocol using 5% NaOCl followed by EDTA on coronal dentin, with and without the application of a universal adhesive used as PEDS. Raman spectroscopy enabled a non-destructive assessment of both mineral and organic dentin components before and after irrigation. The results showed that irrigation without adhesive protection induces measurable alterations in dentin chemistry, including reductions in phosphate-related mineral signals, changes in carbonate substitution, and significant modifications in collagen-related Raman ratios indicative of organic matrix degradation and conformational disorder. In contrast,

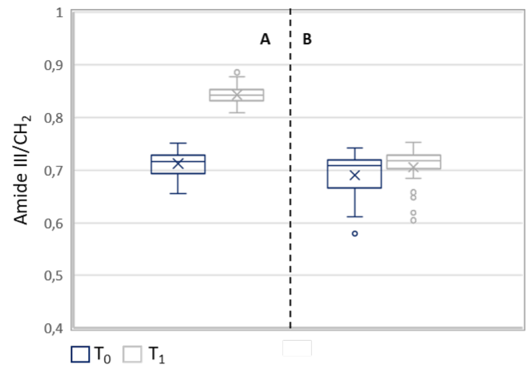

Fig. 4: Boxplot of the Amide III/CH_2 Raman intensity ratio, for Group A (without adhesive) and Group B (with adhesive). Blue boxes represent measurements acquired before irrigation (T0), while gray boxes correspond to Raman measurements performed after the irrigation protocol (T1).

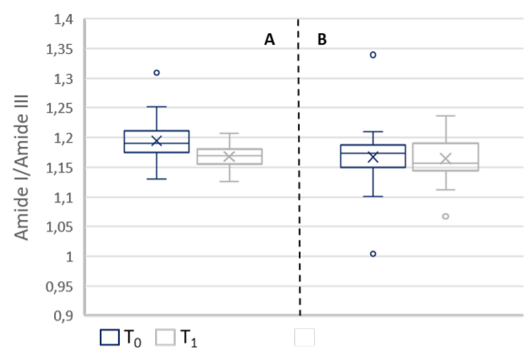

Fig. 5: Boxplot of the Amide I/Amide III Raman intensity ratio for Group A (without adhesive) and Group B (with adhesive). Blue boxes represent measurements acquired before irrigation (T0), while gray boxes correspond to Raman measurements performed after the irrigation protocol (T1).

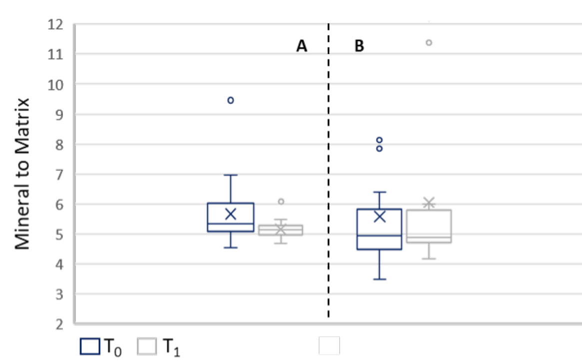

Fig. 6: Mineral to matrix ratio, for Group A (without adhesive) and Group B (with adhesive). Blue boxes represent measurements acquired before irrigation (T0), while gray boxes correspond to Raman measurements performed after the irrigation protocol (T1).

TABLE I: Peak intensities and extracted parameters of dentin mineral and organic components. Values are expressed as Mean (Standard Deviation) and Median [25th–75th percentile] for Group A (without adhesive) and Group B (with adhesive) before irrigation (T0) and after irrigation (T1).

Group	Time	Phosphate	Amide I/Amide III	Amide III/CH2	Mineral-to-Matrix
Group A	T0	32321.71 (10079.44) [32301.09 (25226.81–36922.26)]	1.195 (0.039) [1.190 (1.175–1.211)]	0.712 (0.022) [0.717 (0.695–0.726)]	5.671 (1.077) [5.341 (5.171–5.910)]
	T1	29816.21 (8360.72) [26514.88 (23837.49–36264.62)]	1.169 (0.019) [1.168 (1.156–1.180)]	0.843 (0.018) [0.842 (0.834–0.852)]	5.153 (0.288) [5.149 (4.990–5.290)]
Group B	T0	33671.14 (8556.76) [32085.67 (27601.83–39405.12)]	1.167 (0.058) [1.174 (1.100–1.188)]	0.690 (0.045) [0.709 (0.676–0.718)]	5.589 (2.156) [4.942 (4.942–5.758)]
	T1	32199.92 (8828.38) [29816.33 (27435.11–37155.85)]	1.164 (0.039) [1.156 (1.144–1.190)]	0.705 (0.037) [0.718 (0.702–0.727)]	6.056 (2.794) [4.886 (4.723–5.591)]

specimens treated with PEDS exhibited substantially attenuated changes in mineral-to-matrix and amide-related ratios, indicating effective preservation of both mineral integrity and collagen structure. These findings confirm that the combined action of NaOCl and EDTA affects dentin through demineralization and collagen degradation, which may compromise the quality and durability of the adhesive interface. The application of a universal adhesive before irrigation acts as an effective chemical barrier, limiting irrigant-induced damage and preserving the dentin substrate. Overall, this work provides measurement-based evidence supporting the use of PEDS as a protective strategy during endodontic procedures and highlights the value of Raman spectroscopy as a sensitive tool for evaluating dentin–irrigant–adhesive interactions. Such insights may contribute to the optimization of clinical protocols aimed at improving the long-term stability of post-endodontic restorations.

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