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Comparative Evaluation of Dentin Remineralization Induced by Ion-Releasing Restorative Materials: An In-Vitro Raman Analysis

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Abstract **Purpose:** The research examined the remineralization abilities between three ion-excluding dental restoratives under study: ACTIVA BioACTIVE, BEAUTIFIL Bulk Restorative and Fuji II LC on dentin weakened by demineralization through Raman spectroscopy and Knoop hardness testing (KHN). **Methods:** The investigators used artificially treated dentin specimens to apply test restorative materials as they conducted the study but omitted restorations from the control samples. The analysis through Raman spectroscopy and KHN tests occurred before storage and again at week eight when materials rested inside phosphate-buffered saline. Qualitative material penetration evaluation took place through the use of confocal laser scanning microscopy (CLSM). **Results:** The restorative materials contained in all groups produced an outstanding increase in phosphate Raman peak intensity and KHN measurement when compared to the control group at a significance level of $p<0.001$. ACTIVA BioACTIVE material demonstrated the most notable mean percentage increase of Raman peak intensity reaching 80.93% yet this metric was statistically equivalent to other evaluation groups ($p>0.05$). **Conclusion:** ACTIVA BioACTIVE proved superior to both other restorative materials when it came to dentin remineralization capability. The examined evidence confirms that treating dentin with ion-releasing materials represents a practical approach for minimally invasive dental procedures. **Clinical Relevance:** The materials help protect the tooth structure while simultaneously enabling caries-affected dentin to heal properly.

Key Words Dentin remineralization, Raman spectroscopy, ion-releasing restorative materials, ACTIVA BioACTIVE, Knoop hardness test, minimally invasive dentistry

INTRODUCTION

Dental caries is a high prevalence disease caused by bacteria and the byproducts they produce, that cause demineralization of both enamel and dentin. When applying minimally invasive dentistry, dentists should attempt to prevent, identify and treat early carious lesions. Once caries excavation is indicated, tooth cutting should be minimized based on biological considerations with respect for both the soft and hard tissues and competency in using modern technology and advanced materials. Around 170 million conventional resin composite and dental amalgam restorations are made each year in the United States, however, about 60% of those restorations fail and require replacement [1]. Failure of resin composite restorations is most likely to occur due to secondary caries at the gingival margins of deep carious lesions and in high caries risk patients [2]. The lack of buffering capacity and antibacterial capabilities may contribute to resin composites' increased susceptibility to secondary caries. On the other hand, in cases of deep carious lesions where the remaining dentin thickness is questionable, the use of ion releasing materials as a protective liner before the final restoration with a resin-based composite restoration maybe advisable. This will allow dental tissue remineralization, maintain pulp vitality and preserve the remaining tooth structure [3]. However, the process of dentin remineralization is complex and difficult therefore, several dental restorative materials are purported to provide the prerequisites to facilitate dentin remineralization [4]. The available therapeutic bio-interactive materials are developed as modified resin materials and there is a need to understand their properties, assess their remineralization potentials and identify when using such materials would be preferable to using traditional restorative materials.

Numerous ion-releasing restorative materials are available in the market, these include ACTIVA BioACTIVE-Restorative and BEAUTIFIL Bulk restorative. It has been suggested that these restorative materials continually diffuse calcium, phosphate and fluoride ions. Additionally, they guarantee to stop tooth caries, prevents its recurrence and may recover lost minerals [5,6]. Despite the similarity of these products in providing high influx of ions, they differ greatly in their composition and therapeutic potential. This will consequently affect their physical and mechanical properties [7,8].

The remineralization potential of restorative materials has been evaluated using various ex-vivo techniques such Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and Electron Probe Micro-Analysis (EPMA), these techniques are restricted to mineral quantification on the surface which may not be characteristic of actual remineralization. These approaches are also considered invasive, time consuming and require extensive sample preparation [9,10]. Raman micro-spectroscopy is a useful technique for measuring chemical bonding. Each Raman peak is a distinct identification of a particular vibrational mode for a chemical bond. A Raman peak's intensity varies with the number of molecules present in the scan area, providing a quantitative measure of the molecular presence in the area under examination [11]. This technique has been used in dental material evaluation of a quantified mineral content in several previous studies [3,12,13]. Furthermore, Knoop hardness test (KHN) has been used in research to determine the demineralization and remineralization of tissues. Tissue hardness is a representative to mineral changes as it has been linked to the mineral content of the samples, which may demonstrate a mineral increase or remineralization [14]. Confocal laser scanning microscopy (CLSM) is proved to be a valuable technique for assessing interfacial interactions between the restorative materials and underlying dentin, which is achieved by introducing fluorescent dyes into the bonding or restorative material, that will be subsequently excited by the microscope's laser, causing it to emit light. This illumination allows for a comprehensive examination of material penetration or infiltration, facilitating the study of factors such as density and depth of infiltration [15,16]

Fuji- II LC, BEAUTIFIL bulk restorative and ACTIVA BioACTIVE restoratives were compared for their potential remineralization using optical Raman spectroscopy. The null hypothesis was there are no differences among these materials' remineralization effects on partially demineralized dentin when evaluated with Raman spectroscopy and hardness testing. This study utilized a null hypothesis which stated that Raman spectroscopy and microhardness testing would show no significant difference within the remineralization capabilities of tested restorative materials and the control group. The study assumed that release of ions from materials would show superior remineralization effects compared to the control condition. ACTIVA BioACTIVE together with BEAUTIFIL Bulk and Fuji II LC served as the materials in this study because they are commonly employed clinically and emit different types of ions. ACTIVA BioACTIVE maintains a steady release of calcium, phosphate together with fluoride ions. BEAUTIFIL incorporates surface pre-reacted glass (S-PRG) filler powders and Fuji II LC is designated as a conventional resin-reinforced glass ionomer. The chosen variations represent a suitable framework for comprehensive assessment between materials.

MATERIALS AND METHODS

A total of fourteen human molar teeth were collected after extraction for a previously care-planned reasons. King's College London granted an ethical approval to carry out the study within its facilities through: National Health Service (NHS), Health Research Authority (HRA), the Integrated Research Application System (IRAS ID: 157705) and Research Ethics Committee (REC reference: 16/SW/0220). A written informed consent was obtained from the subjects from whom the teeth were collected. The Spectroscopic and Biomechanical assessments were conducted using Raman spectroscopy and Knoop hardness tester, respectively. Twelve teeth were allocated for these assessments, each tooth being divided into two halves using an EXTEC® Labcut 1010 - Low Speed Diamond Saw (Agar Scientific Limited, EXTEC Corp., Enfield, CT, USA) with water coolant, with each half representing individual samples within their respective groups (N = 24). The samples were divided into four groups, each containing six samples (n = 6). The factorial design consisted of 4 groups x 6 samples in each group x 2 sections (sound and demineralized) for each sample, resulting in 48 tested surfaces for each test (Raman and KHN), including the control. Moreover, two teeth were used for CLSM, with each tooth also divided into two halves which each half representing an individual sample within their respective groups (N = 4, n = 1) and each sample is divided as mentioned previously by a groove into two sections (sound and demineralized). This is to qualitatively visualize and compare the extent and depth of penetration of various ion-releasing restorative materials into dentin surfaces (both demineralized and sound), as well as the general penetration of the solution into unrestored dentin surfaces (sound and demineralized).

Use of blinding procedures was not possible because the restorative materials had distinct physical and visual properties. The set examiner maintained procedural consistency across all measures by performing all assessments. The experimental methodology was carried out outside of the human body which fails to replicate actual oral conditions that include saliva, changing pH levels and bacterial biofilms.

Sample Preparation

Partially Demineralized Dentin

An acrylic mold was then used to fix each individual sample, exposing the cut dentin surface. For the twenty-four samples used in Raman and KHN, a perpendicular

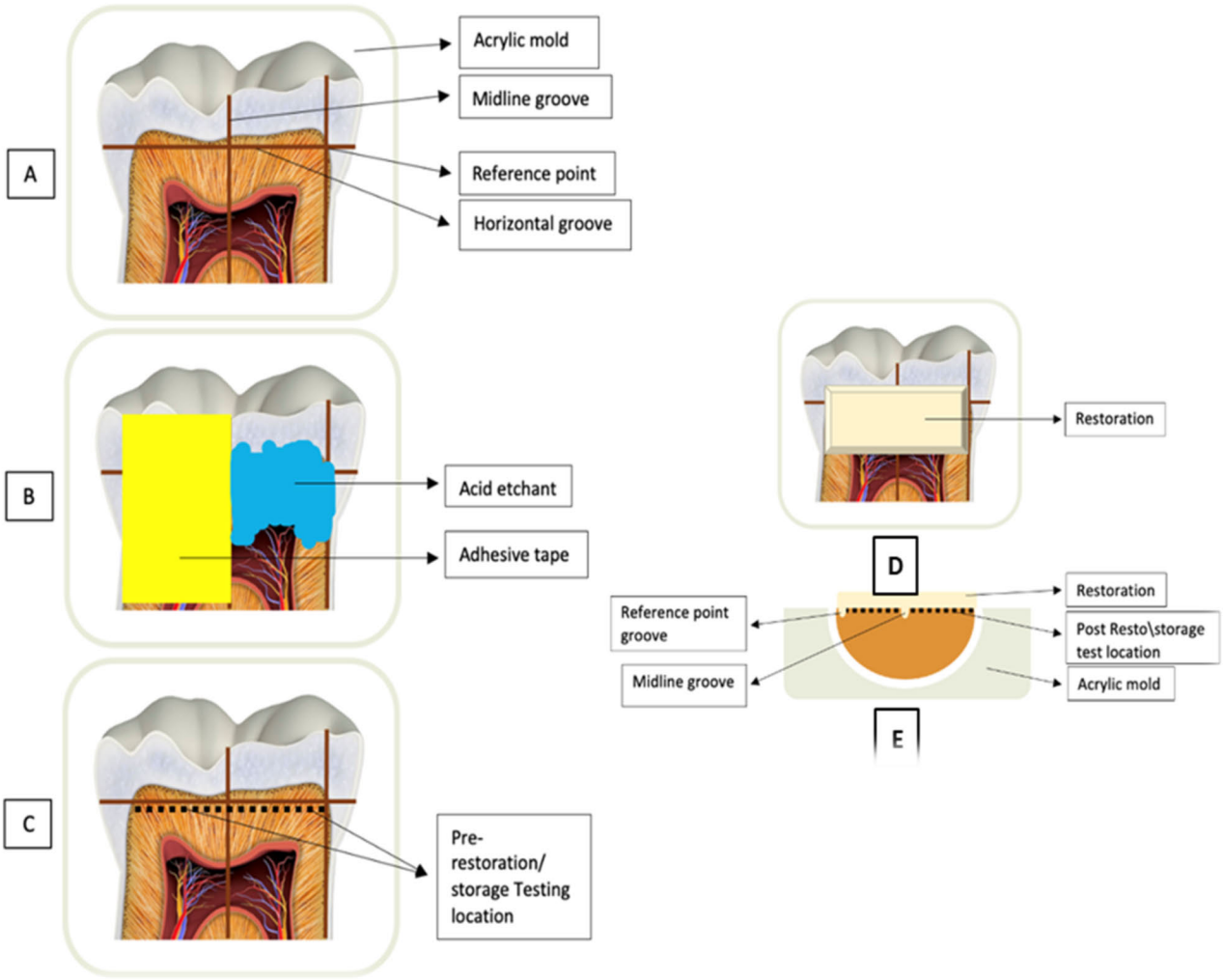

Figure 1(a-e): Illustration of the samples used for testing, (a) The diagram shows midline groove, separating the demineralized and sound dentin sections, a reference point indicating the starting point for testing and a horizontal groove marking the line where the tests will be performed before and after storage, (b) Acid etchant is applied to the section including the reference point to demineralize dentin. The other sound dentin section is protected by adhesive tape, (c) Imaginary dotted line represents the location in dentin where the pre-restoration/storage tests are performed, (d) The restorative materials cover both the demineralized and sound surfaces of the samples and (e) After storage, samples are horizontally cut at the horizontal groove, for spectroscopic and biomechanical assessments are performed along the imaginary dotted line

angle groove was made using the low-speed diamond saw with water coolant at the edge of one proximal dentino-enamel junction (DEJ) to serve as a reference point during measurements. Moreover, a midline groove was created in each half to separate the sound and demineralized sections. Each sample was then polished using sequential silicon Carbide (SiC) abrasive papers (FEPA P # 500, 1200, 2000 and 4000, respectively; Strurers, Champigny sur Marne cedex, France) under running water. (Figure 1a) (Fine Art America, 2023, Available at: https://fineartamerica. com/featured/molar-tooth-cross-section-alfred-asieka.html?product=poster).

One half of each sample was then covered with medical labelling tape to protect the dentin surface, while the other half was subjected to demineralization. This demineralization process was achieved by applying ScotchbondTM Universal Etchant (3 M ESPE, St. Paul, MN, USA; Lot # 6694676) etching gel containing 32 wt.% phosphoric acid for 60 seconds. This resulted in a partially demineralized dentin segment, following a previous protocol [3,17] (Figure 1b). Subsequently, the samples were thoroughly rinsed with deionized water and cleaned in an ultrasonic bath (Fisherbrand® FB15060, Loughborough, Leicestershire, UK) for 5 minutes.

Table 1: The chemical composition, concentration, and manufacturer for the bioactive restorative materials

Material	Commposition	Conc. Weight%	Shade	Manufacturer
ACTIVATM BioACTIVE-RESTORATIVE	Bioactive ionic resin with reactive glass filler: Blend of diurethane and other methacrylates with modified polyacrylic acid. Silica, amorphous. Sodium fluoride.	 44.6% 6.7% 0.75%	A2	PULPDENT® Corporation, Watertown, MA, USA Lot # 220217
BEAUTIFIL-Bulk Restorative	S-PRG filler based on FAS glass. Bis-GMA. UDMA. Bis-MPEPP. TEGDMA. Reaction initiator. Pigments and others.	 60-70% 5-10% 1-5%	Universal	SHOFU INC., Kyoto, Japan Lot # 022259
GC Fuji® II LC CAPSULE	Liquid: Water. 2-hydroxyethyl methacrylate (HEMA). Polybasic carboxylic acid. Dimethacrylate. Urethane dimethacrylate (UDMA). Camphorquinone. Powder: Aluminosilicate glass.	 15-40% 3-7% 1-5% 1-5%	A2	GC Corporation, Tokyo, Japan Lot # 220117B

Samples were then randomly allocated into four groups in (Table 1), with one control group (no restoration). All samples were subjected to both Raman spectroscopy testing and KHN testing before storage as baseline measurements and after storage as explained in the section below 2.2.

Sample Storage

After conducting the pre-restoration/storage assessments for the spectroscopic and biomechanical analysis, each sample was rinsed with distilled water and cleaned in an ultrasonic bath for 5 minutes. Subsequently, groups A (ACTIVA BioACTIVE), B (BEAUTIFIL Bulk restorative) and C (Light-cured resin-reinforced Glass ionomer restorative: Fuji II LC) were applied following the manufacturer's instructions for each specific material. In contrast, the remaining six samples in group D were left as control samples without applied restoration. Following the restorative materials application process, each sample was placed in a separate glass vial containing 7.0 ml of phosphate-buffered saline (PBS) solution (Oxoid Limited, Hampshire, UK). These vials were then kept in an incubator at 37°C for a duration of eight weeks. During this period, the PBS solution was changed twice a week to maintain a conducive environment [3].

For the CLSM samples, the three ion-releasing restorative materials were labeled with Rhodamine (Rd) fluorescent dye (Rhodamine B, Sigma Chemical CO., St. Louis, MO, USA; Lot # 47H3506). The dye was added at a concentration of 0.1 wt.% to each material and thoroughly mixed using a mixing spatula until all the powder was evenly distributed. The dye-incorporated restorative materials were then applied to the samples in groups A, B and C following their manufacturers' instructions. These labeled samples, along with the negative control sample from group D, were stored in separate glass vials containing 7.0 ml of PBS solution in an incubator at 37°C for eight weeks with changing the PBS solution twice a week as described previously.

Post-Storage Sample Preparation

After the completion of the storage period, the samples designated for Raman spectroscopic and biomechanical assessments were cut transversely at the horizontal groove. Samples were then polished and placed in an ultrasonic water bath for 5 minutes as previously described (Figure 1d and e). The number of samples used was determined from previous comparable research articles while Raman and CLSM method feasibility constrained the analysis. A screening process for extracted molars excluded specimens that displayed either previous dental work or caries deterioration or structural defects to achieve standardized testing conditions. To ensure reliability, intra-examiner calibration was performed before testing. The examiner repeated 10 random measurements for both Raman and KHN, achieving over 90% agreement. All samples were handled using sterile tools and stored in sealed containers to minimize contamination.

For the CLSM samples, a transverse cutting technique perpendicular to the restored/exposed surface was utilized. This process involved using the low-speed diamond saw machine with water coolant to create slices of 1.5 mm thickness from each sample. Each sample provided 2-3 slices, which were then polished on both sides as previously

described. Finally, these slices were placed in the ultrasonic water bath for 5 minutes to ensure effective cleaning before further assessment.

Testing

Raman spectroscopy and KHN number assessments were conducted on all samples before and after storage. These tests were performed at both the demineralized and sound sections, just below the horizontal groove (Figure 1c and e).

Raman Spectroscopy (Mineral Peak Analysis)

To analyze the changes in the phosphate mineral peak between sound and demineralized areas within the samples, a Confocal Raman microscope (Renishaw, inVia™ Raman Microscope, New Mills Gloucestershire, UK) with a 785 nm diode laser and 6000 1/mm gratings was utilized. The standard confocality was employed in Map Image Acquisition scanning mode for data collection. The samples were imaged using a 20x 0.40 numerical aperture (NA) air objective with a Renishaw CCD camera to locate the reference point and the midline groove (Figure 1c). The grating scan type was extended, covering the spectrum range from 850 to 1050 cm^{-1}. During acquisition, the settings were as follows: 10 seconds exposure time, 10% laser power and an accumulation of 1. For the demineralized section scan, a line scan was conducted, commencing from the reference point and extending to the midline just below the horizontal line. On the other hand, for the sound section scans, the line scan started at the cross-point between the horizontal and midline grooves and it extended through the dentin until reaching the DEJ just below the horizontal line (Figure 1c). To ensure sufficient data points, the number of steps was adjusted to perform 10-12-point scans along the line scan for each section (sound and demineralized), which correspond to a reading every 0.25 mm. The obtained scan results were then processed by subtracting the baseline for each scan using the processing tool in the software. The data were saved in an XML-based format, which was later opened as an Excel file for further analysis. The phosphate peak intensities around 959 cm^{-1} were measured in both the sound and demineralized areas. The percentage change in the mineral peak before and after storage was calculated for each sample then the mean intensity was calculated separately for each group, considering sound and demineralized dentin separately.

Biomechanical Assessment of Tissue Hardness

The hardness testing was conducted using a Struers DurScan 20 G5 microhardness tester (Kemet International Limited, Kent, UK). A KH 0.1 force (100 grams load) was applied for 15 seconds and the observations were made at X10 magnification with X2 zoom level. For the demineralized section, the first indentation was placed at the reference point. Subsequently, indentations were made at 0.5 mm intervals along a line parallel to the horizontal groove, just beneath it and continuing until reaching the midline groove. At the sound section, the first indentation in the dentin was made just after the midline groove. From there, further indentations were performed at 0.5 mm intervals along a line just below the horizontal groove, extending until reaching the DEJ. The software cursors were adjusted at the ends of the rhombus-shaped indentations and the hardness number for each tested area was then automatically calculated by the manufacturer's software and recorded.

CLSM Assessment

CLSM is utilized to assess and compare the extent and depth of penetration of different ion-releasing restorative materials, as well as their leaching into the restored dentin surfaces (both demineralized and sound) qualitatively visually. Additionally, it allows comparison with the general penetration of the solution into unrestored dentin surfaces (both sound and demineralized). For analysis, the restoration-dentin interfaces of groups A, B and C, as well as the unrestored, previously exposed dentin surfaces for both the demineralized and sound sections, were examined using an inverted CLSM (Nikon Eclipse Ti2 Inverted, Minato, Tokyo, Japan). A 40X lens was employed and emission fluorescence at 585-650 nm for Rd was utilized. The images were viewed using NIS Elements Viewer software (Version 4.11.0).

Statistical Analysis

To assess the effectiveness of the dentin demineralization protocol, the differences between sound and demineralized dentin were compared for the Raman-mineral peak and tissue hardness measured by KHN number. The percentage change between pre- and post-storage readings was calculated for both sound and demineralized sections to explore significant differences among the different material groups (ACTIVA BioACTIVE, BEAUTIFIL, Fuji II LC and No restoration) for each assessment method. Statistical analysis was utilized to determine significant differences between the groups (sound and demineralized groups) for each technique (Raman and tissue hardness) and material (ACTIVA BioACTIVE, BEAUTIFIL, Fuji II LC and control). The normality of the data was assessed using Shapiro-Wilk's test, revealing that the data were not parametric. Consequently, the Kruskal-Wallis test was applied for intergroup comparisons, followed by Dunn's post hoc test. For intragroup comparisons, the signed rank test was used. To address multiple comparisons, p-values were adjusted using the Bonferroni correction. The significance level was set at $p<0.05$ for all tests. R statistical analysis software version 4.3.1 for Windows was utilized for performing the statistical analysis. The researchers employed both effect size calculations (Cohen's d) and 95% confidence intervals together with Kruskal-Wallis and Dunn's post-hoc tests for important comparisons. The reported metrics enabled researchers to comprehend real-world implications of the group difference findings.

RESULTS

Spectroscopic and Biomechanical Assessments for Remineralization Potential

Pre-storage Raman mineral peak intensity and KHN values for the demineralized dentin sections were found to be statistically significantly lower than the sound dentin sections ($p<0.001$) (Table 2).

Table 2: Comparison between the sound and demineralized samples before storage for both the Raman peak and KHN measurements

Measurement	(Mean ±SD)		u-value	p-value
	Sound	Demineralized		
Raman peak	19754.24±2115.92	9509.48±1470.68	30604	<0.001*
KHN	47.86±4.74	33.71±3.45	9591	<0.001*

*Significant (p<0.05)

Table 3: Intergroup and intragroup comparisons of Raman peak values percentage change for and between the demineralized and sound dentin

Samples	(%) (Mean ±SD)				h-value	p-value
	Group (A)	Group (B)	Group (C)	Group (D)		
Demineralized	80.93±30.94A	77.66±27.69A	83.40±41.59A	13.07±108.45B	106.96	<0.001*
Sound	0.49±6.05A	0.66±13.31A	-14.14±118.70A	-13.56±103.92A	1.78	0.619
u-value	1805	1980	1820	709		
p-value	<0.001*	<0.001*	<0.001*	0.776		

Different superscript letters indicate a statistically significant difference within the same horizontal row, *Significant (p<0.05)

Table 4: Intergroup and intragroup comparisons of KHN values percentage change for and between the demineralized and sound dentin

Samples	(%) (Mean ±SD)				f-value	p-value
	Group (A)	Group (B)	Group (C)	Group (D)		
Demineralized	47.29±17.59A	37.67±15.66A	40.44±17.17A	6.88±19.16B	37.51	<0.001*
Sound	-3.92±18.18A	-0.98±13.65A	1.46±13.88A	-3.23±16.70A	0.82	0.483
t-value	12.12	9.71	9.77	2.57		
p-value	<0.001*	<0.001*	<0.001*	0.015*		

Different superscript letters indicate a statistically significant difference within the same horizontal row; * Significant (p < 0.05)

Post-storage, intergroup comparisons of the Raman peak intensity and KHN values percentage change for the demineralized dentin were statistically significant lower within the control group (D) only (p<0.001) compared with the high percentage change in the other groups. However, there was no statistically significant difference in the percentage change between the other groups of the demineralized dentin (A, B and C) or between the groups of the sound dentin for wither the Raman peak (p = 0.62) or KHN (p = 0.48) values (Table 3 and 4) and (Figure 2).

The intragroup comparison between the sound and demineralized sections of the Raman peak intensity shows no significant difference in the percentage change within the control group (D) (p = 0.776). However, within the other restorative groups (A, B & C); the percentage change of the Raman peak intensity within the demineralized dentin was statistically significantly higher than that of the sound dentin (p<0.001) (Table 3) and (Figure 2a and b).

The intragroup comparison between the sound and demineralized sections of the KHN shows that the percentage change for the demineralized dentin was statistically significantly higher than that of the sound dentin within all material groups (p<0.001) and for the control group D (p = 0.015) (Table 4) and (Figure 2a and b).

Qualitative Assessment of the Extent and Depth of Penetration into Demineralized and Sound Dentin

After examining the confocal images for the sound and demineralized sections of the four groups (Figure 3), it becomes apparent that ACTIVA BioACTIVE exhibits denser and deeper penetration and extension into both sound and demineralized dentin in comparison to the other ion-releasing restorative materials. Moreover, the penetration pattern of ACTIVA BioACTIVE more closely resembles that of the negative control group without restoration.

DISCUSSION

ACTIVA BioACTIVE outperforms other materials because of its special resin matrix design that maintains continuous ion exchange. The material shows the ability to release fluoride calcium and phosphate ions which helps create hydroxyapatite-like structures for better dentin mineralization. This study presented a non-invasive assessment of mineralization comparing two ion-releasing resin restorative materials on artificially demineralized dentin. The null hypothesis was accepted as there were no significant differences in the mineral peak or hardness number of tested dentinal substrates when stored with the investigated materials. However, highly significant differences were found when these materials were compared with the control group (no restoration).

Natural variations in the mineral content of carious dentin lesions and the extent of organic matrix breakdown make its assessment more challenging [18]. Therefore, the used methods independently quantify the mineral content of dentinal tissues. This might offer a more precise understanding of the tested materials' efficacy and provide a useful model for comparing different materials regarding their remineralization potentials [3,10]. The mineral content of dental tissues is directly proportional to the relative dentin micro-hardness measures, which were found to be linked with the Ca:P ratios of dentin [10]. The hardness number (KHN) were previously established for soft and firm carious dentin by Almahdy *et al.* [14]. These values were used in this study as a reference to validate a representative caries affected dentin model and possible change in the mineral content, hence assess the remineralization potential of the investigated materials [3,14]. Moreover, a correlation between the mineral changes detected by Raman spectroscopy and micro-hardness values was observed in this study. A significant increase was found in the intensity of apatite's phosphate

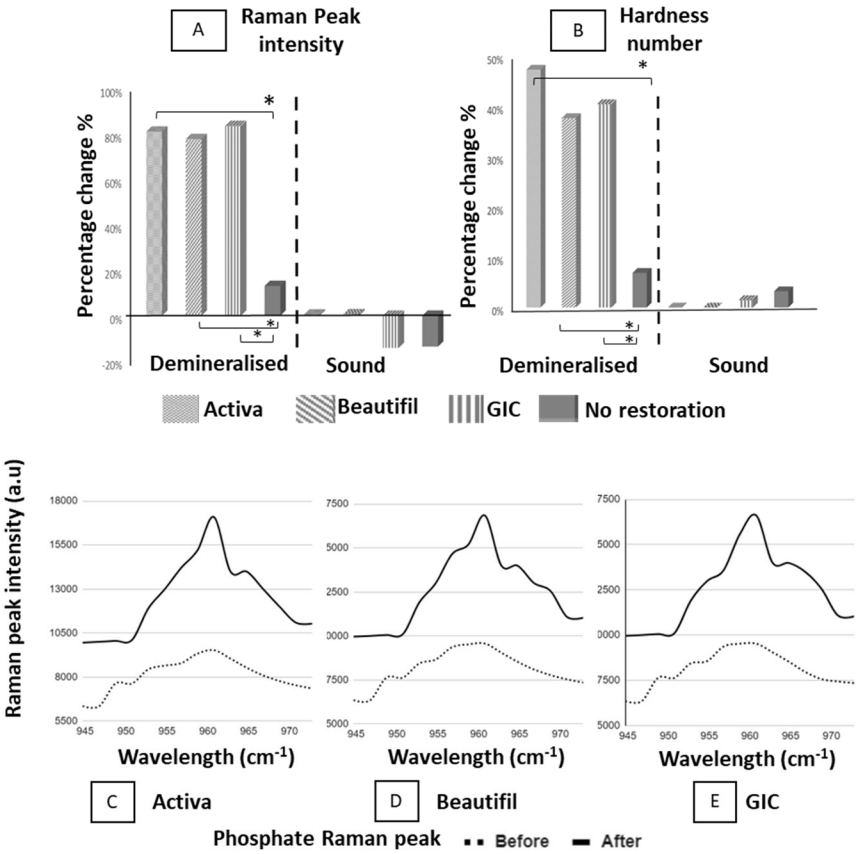

Figure 2(a-e): The percentage change for the demineralized and sound dentin of each group for: (a) Phosphate Raman peak, (b) Microhardness KHN values, (c-e) Characteristic phosphate Raman peak (959 cm^{-1}) of demineralized dentine before and after storage in PBS, where: (c) ACTIVA BioACTIVE, (d) BEAUTIFIL, (e) Fuji II LC, *Represents statistically significant difference with a p-values <0.05

Raman peak ($PO4^{-3}$ v1) at 959 cm^{-1} and the measured hardness of these substrates before and after treatment (Figure 2c and e).

This Raman peak has been used to determine the mineral content of dentin [14] and to verify if the tested materials enable apatite precipitation [19,20]. The V1 band in the Raman spectra of fluorapatites matches that found in hydroxyapatite, which was utilized in this study's peak analysis [21]. ACTIVA BioACTIVE composite had a slightly higher percentage change than BEAUTIFIL and Fuji II LC when evaluated on partially demineralized dentin.

Earlier in-vitro investigations used a variety of protocols to achieve demineralized dentin, the most popular and simple of which used phosphoric acid. As shown in (Figure 2a and b), a significant difference was measured between sound and demineralized values for both the phosphate Raman peak and hardness, which confirms the mineral loss in the demineralized samples. Moreover, this demineralization protocol guaranteed that the collagen is not completely destructed and it represent caries affected dentin [3]. The presence of collagen is crucial when evaluating the potential of remineralization, as these materials are only effective on partially demineralized dentin by relying on pre-existing mineral crystals and use them as nuclei for mineralization and repairable collagen matrix with available non-collagenous proteins to regulate the remineralization process [17,22].

The current study compared available ion-releasing restorative materials in term of their remineralization potential: ACTIVA BioACTIVE, BEAUTIFIL and resin-reinforced glass-ionomer restorations. These materials share the same clinical indications and somehow similar compositions including resin matrix and Glass Ionomer Cement (GIC) component that releases fluoride and form fluorapatite following the classical remineralization pathway [23]. Although without significant differences, ACTIVA BioACTIVE- Restorative showed the highest increase in both mineral peak and microhardness. In addition to the fluoride release, ACTIVA BioACTIVE has previously shown to release a significant amount of calcium and phosphate ions which may facilitate slight improvement in mineral deposition [6,24]. The ion release and deposition from ACTIVA BioACTIVE was investigated in earlier studies and concluded that the resin matrix component in the material provide a reservoir for ions which allow for higher and long-term release of both fluoride and phosphate than other materials such as GIC [25,26]. Both studies agree with the findings in this investigation. This was also reflected in the slightly higher KHN number of demineralized tissues

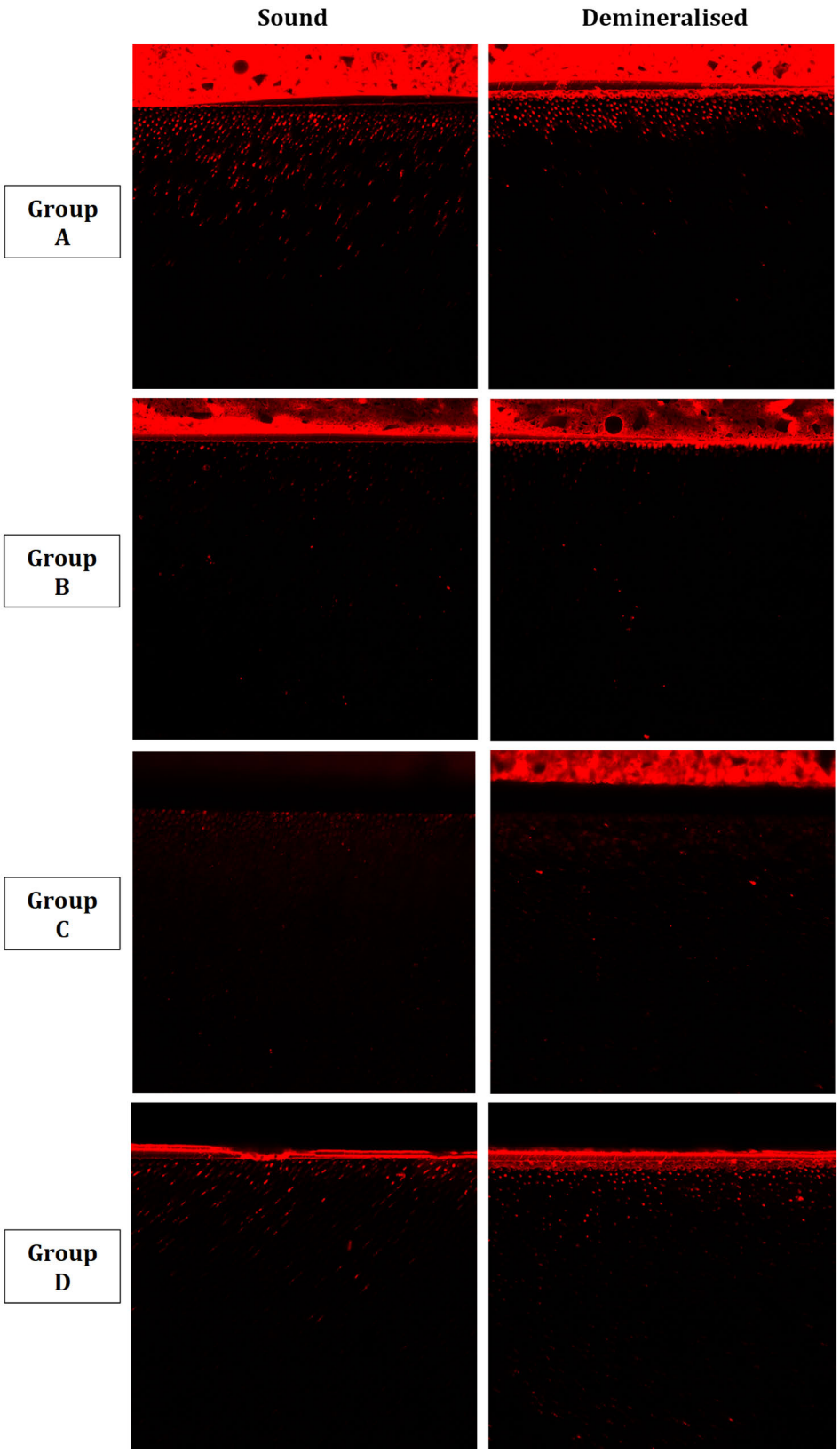

Figure 3(a-d): CLSM images depicting the restoration-dentin interface for groups A, B, and C, as well as the non-restored group D sections for both the sound and demineralized dentin. The ion-releasing restorative materials in the restored groups and the PBS storage solution in the non-restored group were labeled with Rd fluorescent dye for visualization. The left column images correspond to the sound section, while the right column images pertain to the demineralized section. Each row represents images for each respective group

with ACTIVA BioACTIVE material compared to Fuji II LC and BEAUTIFIL. On another study, the higher ion release of ACTIVA BioACTIVE explained the better marginal seal and decreased postoperative hypersensitivity found in clinical evaluation of this material [27]. Fuji II LC releases fluoride ions mainly, with traces of other ions. This displayed a higher mineral peak and hardness than BEAUTIFIL. Similar results were previously reported when different types of GIC was compared to ion-releasing restorative materials, fluoride release was found higher from GIC with an increase the hardness number of the samples [28,29]. BEAUTIFIL restoration is composed of bioactive surface pre-reacted glass that releases six different ions. Fluoride is among these ions which is released in high amount with the ability to recharge and release in a similar manner to GIC [25,30]. Studies demonstrating the release of calcium and phosphate ions following BEAUTIFIL Bulk restoration are limited. However, Fluoride discharged from BEAUTIFIL was reportedly lower than that from other glass ionomer materials which explains the decreased mineral peak and KHN in this study's samples after storage [31].

All groups showed a penetration from the dentin surface into the dentinal tubules. However, due to the qualitative nature of the implied CLSM testing, more tests need to be performed to quantitatively measure the depth of penetration and consequently, the depth and extent of action of the ion-releasing material into dentinal tissue.

The current study aimed to compare three ion-releasing restorative materials using an *in vitro* demineralized dentin substrate. This may not specifically simulate the natural process of biomineralization that occur in the oral environment. However, the proposed in-vitro method might offer an approximate understanding of these materials' performance as well as a useful model for comparing restorative materials regarding their remineralization potentials. Another limitation on this in-vitro protocol was its attempt to replicate physiological settings; while our basic dentine remineralization model was suggestive, it was unable to capture all the nuances of the in-vivo process. Therefore, It is recommended to perform further in vitro investigations including natural carious dentin as a substrate as well as additional clinical trials to assess dentin remineralization using ACTIVA BioACTIVE and BEAUTIFIL restorations on carious teeth after excavation. A final limitation of this study is that the depth of demineralization and remineralization was not calculated. For future studies, it may be useful to utilize Raman spectroscopy for obtaining additional details and mapping of the hybrid layer. This approach can provide more information on the extent of infiltration and the subtle biochemical changes within the hybrid layer. Moreover, Raman can be utilized to include organic matrix evaluation along with a comparison of the materials effect at different depth within affected dentin. The clinical use of ACTIVA BioACTIVE makes the most sense when treating deep carious lesions because dentin preservation stands as a primary consideration. Clinical application as a liner or final restoration can help preserve tooth vitality thus minimizing the requirement for deep dental tissue elimination in minimal treatment approaches. The research model operated in a test tube environment without examining product durability when exposed to changes it would encounter in the mouth. The sensitivity levels of Raman spectroscopy measurements for mineral content might be affected by both background noise and surface topography. The small number of samples tested makes it difficult to apply research results to a wide range of scenarios.

CONCLUSIONS

Limitations of this in-vitro research showed all tested ion-releasing materials positively affected the mineral structure and strength features of dentin which experienced partial demineralization. The mean percentage changes across Raman peak intensity and KHN measurement exceeded those of BEAUTIFIL and Fuji II LC although the results were not statistically different. The research indicates that ACTIVA BioACTIVE delivers improved remineralization properties which establishes it as a strong candidate for modern restorative approaches. Additional research demands clinical trials and lasting performance tests together with evaluations of salivary pH and microbial biofilm effects on remineralization abilities.

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