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Title:

Effect of CPP-ACP Modified-GIC on Prevention of Demineralization in Comparison to Other Fluoride-Containing Restorative Materials

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Abstract

Background: This study evaluated the ability of a CPP-ACP-modified GIC to inhibit demineralization around the margin of cervical cavities in natural teeth in comparison with a Giomer and conventional GIC with and without coating.

Methods: Thirty-two sound human molars were used. Box-shaped cavities were prepared along the CEJ. Teeth were randomly divided into 4 groups and restored with Equia Forte Fil, Coated Equia Forte Fil, Fuji VII EP, or Beautifil II. Teeth were subjected to pH cycling. Micromorphological and elemental analyses were done using SEM and EDX. Polarized light microscope analysis and microhardness tests were also performed.

Results: Microhardness tests on enamel showed a significant difference between the coated Equia group, Equia, and Beautifil II groups ($p<0.05$). Dentin results showed significant differences between the coated Equia group and all other groups ($p<0.05$). Elemental analysis showed significant differences in calcium weight percentage among the first and second observation levels in all groups ($p<0.05$). A significant difference was found between the coated Equia group and the other three groups ($p<0.05$).

Conclusions: All tested materials showed some ability to resist demineralization at the restoration margins. The coated GIC restoration showed better outcomes compared with the other tested materials.

Keywords

CPP-ACP, GIC coat, Giomer, Glass ionomer, Remineralization

Introduction

Glass ionomer cements (GIC) were first developed to introduce a material that could combine matching the properties of and adhesion to tooth structure, as well as having a limited effect on the pulp.[1, 2] GIC is a unique material which can chemically bind to the calcium ions of hydroxyapatite in tooth structure via the formation of ionic bonds.[3] GICs exhibit anti-cariogenic properties attributed to the release of fluoride. Furthermore, they have been shown to exhibit antimicrobial effects as well as influence plaque formation.[4] Current GICs reach an equilibrium of a low level of ion release that may not be enough to prevent demineralization of tooth structure with frequent exposure to acids from the biofilm. For these reasons, the development of new

materials or modification of current materials has been conducted to enable more reliable anti-cariogenic effects. These materials are characterized by the enhanced release of fluoride and/or other ions, such as calcium and phosphate, which may help in remineralization or prevent demineralization after acid exposure.

The incorporation of CPP–ACP into a conventional GIC powder is a recent modification of GIC.[5] CPP–ACP was first incorporated into GIC powder in 2003 by Mazzaoui et al.[6] Casein phosphopeptide (CPP) is produced by the aggregation of calcium phosphate with the milk protein casein's tryptic digestion followed by purification using ultrafiltration.[7] The CPP can stabilize calcium phosphate in solution leading to an elevation of calcium phosphate levels in dental plaque adjacent to tooth surfaces. Moreover, CPP prevents the growth of amorphous calcium phosphate (ACP) clusters to the size needed for nucleation and precipitates by binding with multiple phosphoseryl residues of CPP.[7] The anti-cariogenic effect of the CPP-ACP was attributed to localization of ACP in dental plaque, buffering the free calcium and phosphate ion activities, thereby helping to maintain a state of super-saturation of Ca and PO_4 ions.[7] Many studies have shown that CPP-ACP has a strong anti-cariogenic effect and can remineralize subsurface lesions in human tooth enamel.[7, 8] A recent paper[9] showed that topical application of CPP-ACP/F paste to Fuji VII EP could enhance ion release. This may increase acid resistance and impede caries initiation and progression.

Recently, a new modified glass ionomer, EQUIA Forte Fil (GC Corp), has been introduced.[10] This GIC is reinforced by two mechanisms, one is the uniform distribution of ultrafine highly reactive glass particles, and the other is increasing the molecular weight of the polyacrylic acid. This new GIC is characterized by improved mechanical properties. The manufacturer claims that this new GIC can be used in load-bearing posterior approximal restorations in addition to occlusal and cervical restorations.[10] Later studies[11, 12] reported that this modified GIC has higher flexural strength and surface hardness, and compressive strength compared to previous conventional GICs. These properties might enable this material to be used in a wide range of clinical applications in dental practice.[11, 12]

Glass ionomer cement is known for its sensitivity to water exposure during the setting process, particularly the conventional GICs. This short-term sensitivity can adversely affect the mechanical properties of the material, as well as aesthetics.[13-15] In order to overcome this problem, the recommendation has been to coat the surface of the GIC with either Copal™ varnish, a proprietary varnish, light-cured bonding resins, or petroleum jelly.[2, 16-18] This coat

preserves the mechanical properties as long as it remains on the restoration.[19] Currently, a new resin coating for conventional GICs has been developed which is characterized by the addition of nanofiller particles. Equia Forte coat (GC Corp, Japan) is a highly dispersed nanofilled resin coating that was introduced to improve the marginal sealing of the GIC and increase its wear resistance.[20] Several laboratory tests have reported a positive impact of this coating on the fracture strength and reducing early wear.[21-23]

Giomer is a modified resin composite that has the addition of pre-reacted glass (PRG) ionomer filler particles within the resin matrix.[24, 25] These particles provide giomers with the potential to promote tooth remineralization, and at the same time, its composition allows it to possess the physical and aesthetic properties comparable to conventional resin composite restorative materials. Several studies conducted on giomers revealed that it has a good clinical performance.[26-29] Yap et al.[30] found that, after polishing, giomer exhibited a better surface smoothness compared with glass ionomer cement. The second generation of giomer has excellent properties as a result of the development of the filler structure. This development allows it to simulate the optical properties of natural tooth structure.[31] A study assessed the ability of Giomer II to maintain fluoride release, fluoride recharge, and mechanical stability over 18-months aging and concluded that it has the ability to sustain intrinsic fluoride release and maintain fluoride recharge capability even after long-term aging. In addition, Giomer II is capable of maintaining its mechanical properties even after long-term water aging, this includes its fluoride release and fluoride recharge capabilities.[32] It has also been reported that Giomer II had a superior surface finish compared to a resin-modified glass ionomer (Fuji II LC, GC Corp, Japan) in non-carious cervical lesions in a one-year clinical study.[33]

Current evidence for CPP-ACP-modified GIC and Giomer II concerning the prevention of demineralization remains limited. In addition, all previous one-arm studies were conducted to compare either CPP-ACP-modified GIC or Giomer with conventional GICs. Thus, the present laboratory study was conducted to evaluate the ability of CPP-ACP-modified GIC to inhibit demineralization around the margin of cervical cavities in natural teeth in comparison with a Giomer II and conventional GIC. Furthermore, evaluation of whether the protective coating of the recent glass ionomer cement shows any effect when subjected to an acid exposure simulating demineralization similar to dental caries.

Materials and methods

Compliance with Ethical Standards

The experimental and human teeth collection protocols were approved by The University of Melbourne Human Research Ethics Committee "under ID number 1647318.1".

All procedures performed in the study involving human parts (extracted teeth) were in accordance with the 1964 Helsinki declaration and its later amendments.

Informed consent: Informed consent was obtained from all individual participants to use their extracted teeth which were included in the study.

The materials used in this study are listed in Table 1.

Tooth selection

Thirty-two freshly extracted sound human molars were collected. Teeth were free from caries, restorations, cracks, or other defects. All selected teeth were cleaned under running water then an ultrasonic scaler (Meta Dental Com, Korea) was used to remove any remaining soft tissue or hard calculus deposits. Selected teeth were then stored in 0.5% chloramine T at 4˚C for 2 weeks then stored in 4˚C distilled water containing crystals of thymol to prevent bacterial growth until use.

Cavity preparation and restorative procedures

The buccal surfaces of each tooth were cleaned. Box-shaped cavities 3 mm long × 4 mm wide × 2 mm deep were prepared along the cervical area of the crown. A second cavity was prepared on the root surface of each tooth with the dimensions of 3 mm long × 2 mm wide × 2 mm deep. Cavities on the root surface were prepared after removing the cementum layer using finishing burs to expose the dentin surface. Cavities were prepared using a parallel-sided bur (Komet Brasseler, Lemgo, Germany) in a high-speed handpiece with air-water spray. The prepared teeth were randomly divided into 4 groups (A, B, C and D). In groups A, B and C the prepared cavities were initially conditioned with 20% polyacrylic acid for 10 seconds then rinsed and gently dried. Teeth in groups A and B were restored with Equia Forte Fil. In Group B, restorations were coated by Equia coat after finishing and polishing, then light-cured with an LED curing light (Radii Plus, SDI, Bayswater, Australia, 1500 mW/cm^2 output). Fuji VII EP was placed in group C. Beautifil II was used in the final group (D) after bonding using FL bond II (two-step self-etch adhesive). All restorative materials were applied according to the manufacturer's instructions.

All specimens were stored for 24 hours at 37˚ C and 100% relative humidity, then finished and polished with a slow-speed handpiece at 6000 rpm using polishing discs (Sof-Lex discs; 3M,

USA). The integrity of the margins of each restoration was verified under a dissecting microscope at 25× magnification. Acid-resistant nail varnish was applied to cover the tooth surface leaving a 1 mm gap around the cavity margins. Teeth were then subjected to pH cycling.

Demineralizing/remineralizing cariogenic challenge (pH cycling)

A demineralizing solution was prepared using 50 mmol/L acetic acid, 1.5 mmol/L $CaCl_2$ and 0.9 mmol/L KH_2PO_4 adjusted to pH 5.0 with KOH. Also, a remineralizing solution consisting of 1.5 mmol/L $CaCl_2$, 0.9 mmol/L KH_2PO_4, 139 mmol/L KCl and 20 mmol/L HEPES buffer and adjusted to pH 7.0 with KOH was prepared.[34]

Fresh solutions were prepared every 24 h for each phase of the demineralization/remineralization cycle. Each specimen was immersed in the demineralizing solution for 14 h, and the remineralizing solution for 8 h to mimic high caries risk conditions.[34] The pH cycling was performed at 37 °C for 15 days. The specimens were rinsed thoroughly with deionized water between each change of solution. Upon completion of the pH cycling, specimens were thoroughly washed with deionized water.

After completion of the demineralizing/remineralizing challenges, all the specimens were embedded in a self-cured epoxy resin. After setting, the specimens were longitudinally sectioned through the center of each restoration using a Minitom (Struers, Copenhagen, Denmark) low-speed water-cooled diamond saw. One half was used for polarized light microscope analysis and the other half for microhardness, SEM and EDX analyses.

Changes in color birefringence of remineralized and demineralized tooth structure using polarized light microscopy (PLM):

Longitudinal sections approximately 300 μm thick were initially obtained then further polished on a water-cooled polishing unit (TegraPol 21, Struers, Copenhagen, Denmark) to a thickness between 80-100 μm with a series of silicon carbide abrasive papers finishing with 4000-grit. Each section containing a lesion formed in enamel and dentin was placed on a microscope slide, imbibed with water, and covered with a coverslip. The slides were visualized at ×200 magnification with a crossed polarized microscope before being photographed. Changes in color birefringence of the specimen were recorded using a first-order red plate between crossed polarizers and enamel placed in the negative quadrant with transmitted light microscopy

(DM2000, Leica Microsystems GmbH, Wetzlar, Germany) coupled to a camera (Leica EC3, Leica Microsystems GmbH, Wetzlar, Germany).

Cross-sectional microhardness analysis

The surface of each section was polished on a water-cooled polishing unit (TegraPol 21, Struers, Copenhagen, Denmark) with abrasive papers (400-, 600- 1200-, 2000-, and 4000-grit), followed by 1 µm diamond pastes (Kemet ™, Type K, Kemet International Ltd., Maidstone, UK). Cross-sectional microhardness measurements (402 MVD, Wolpert Wilson Instrument, MA, USA) with five indentations from the tooth surface towards the pulp were performed, spaced 100 µm apart and at distances of 100, 250, 400, and 550 µm from the restoration margin. The parameters for indentation of Knoop hardness indentation (KHN) were a 25-g load and 10s dwell time.[35] Hardness was determined from the following formula:

$$H = \frac{\mathrm{P}}{\mathrm{A}}$$

where P is the applied load and A is the contact area of the indentation which depends on the indenter geometry and the penetration depth.[36]

Micromorphological and elemental analysis using scanning electron microscopy (SEM) and energy-dispersive x-rays (EDX).

The cut surfaces were polished with silicon carbide papers and diamond pastes as described above. The specimens were then ultrasonically cleaned for 1 min in distilled water. The bonded interface was subjected to an acid-base challenge using 10% ortho-phosphoric acid for 10s and then washed with distilled water and immersed in 5% sodium hypochlorite solution for 5 min, then finally rinsed in distilled water. The specimens were sputter-coated with a platinum/palladium alloy (Magnetron sputter MSP-2S; IXRF system Inc., Austin, TX, USA) and observed with a scanning electron microscope (FEI Quanta SEM, FEI, OR, USA) at 15.0 kV. Micromorphological analysis of the restoration/tooth interface was done at magnifications of 500×, 2000×, and 5000 ×.

Uncoated specimens were subjected to EDX analysis using Aztec 3.3 software (Oxford Aztec) to determine the elemental content of the enamel. Elemental analysis was performed under low vacuum at 1000× magnification using EDX attached to the SEM. The recorded values represented the average mineral weight percentages.

Statistical analysis

Data were tabulated using Microsoft excel then subjected to statistical analysis using the statistical package for social science (IBM-SPSS) version 23.0. Data were expressed as means ± standard deviation. The normal distribution of data was checked by the Kolmogorov-Smirnov test and Shapiro-Wilks test. Homoscedasticity was checked by Levene's test which showed no significance ($p>0.05$). Statistical analysis of the microhardness and elemental analysis data were carried out by two-way ANOVA and Tukey's post hoc for multiple comparisons. P-value <0.05 was considered statistically significant.

Results

Microhardness

Results of the microhardness test on enamel (Table 2) showed that enamel microhardness was significantly greater for Coated Equia group compared with the Equia and Beautifil II groups ($p<0.05$). There was no significant difference between either Fuji VII EP, Equia groups or Fuji VII EP and coated Equia groups ($p>0.05$). Also, no significant difference in microhardness was found among different indentation levels from restoration margins within the same group ($p>0.05$).

The results of the microhardness test on dentin (Table 2) showed that the results of the Coated Equia group were significantly greater than all other groups ($p<0.05$). No significant difference was found among Fuji VII EP, Equia fortefill, and Beautifil II groups ($p>0.05$). No significant differences in microhardness were found between different levels in the same group.

Detection of changes in color birefringence of remineralized and demineralized tooth substrate using polarized light microscopy (PLM)

Changes in birefringence of the specimens showed the presence of a remineralized layer next to the restorative material in Equia Fortefil and Fuji VII EP groups which indicates their remineralizing/protection effect in the region adjacent to the restorative material (Fig. 1 and 2). No remineralization was noted for Coated Equia Fortefil, this was believed due to the protection of the coating (Fig. 3 and 4).

Micromorphological analysis of restoration/tooth interface using Scanning Electron Microscopy (SEM):

Micromorphological analysis of dentin/restoration interface revealed good adaptation between the Fuji VII EP, Equia Forte Fil and dentin. An acid-base resistant layer was observed in all groups which is an indication of the ability to resist demineralization. A hybrid layer was identified between the bonded dentine and FL Bond (Fig. 5).

Elemental Analysis using Energy Dispersive X-Ray Analysis (EDX):

The outcome of the elemental analysis is presented in Table 3. In each group, the first observation level (50 μm from the restoration interface) was significantly higher in calcium weight fraction than the second observation level (150 μm from the restoration) in all groups ($p<0.05$). The Coated Equia group had significantly higher calcium weight fractions than the other three groups ($p<0.05$) at the 150 μm level.

Regarding phosphorus weight fraction results, the first observation level (50μm from the restoration) showed significantly higher amounts than the second (150μm from the restoration) in the same group ($p<0.05$) except for the Coated Equia group. The coated Equia group showed significantly higher amounts of phosphorus than both Equia Forte Fil and Beautifil II groups ($p<0.05$) at the 150 μm level. No significant difference was found between Fuji VII EP and the other three groups ($p>0.05$). Regarding the Ca/P ratio, no significant difference was found among different groups ($p>0.05$).

Discussion

Glass ionomer cements have been referred to as "smart materials" due to their ion exchange, recharge properties from the external environment, chemical adhesion to the tooth structure as well as the ability to release more ions when exposed to an acid.[37, 38] Several laboratory studies have demonstrated the potential of CPP/ACP to enhance remineralization of tooth structure.[39] Calcium phosphopeptide is supposed to stabilize free Ca/P ions through binding amorphous calcium phosphate and forming CPP/ACP clusters.[8] These clusters can bind to dental plaque[40] and act as a calcium phosphate reservoir that can maintain a supersaturated mineral state

at the tooth surface. On acid challenge, CPP/ACP clusters can inhibit demineralization and enhance remineralization.[41, 42]

The area of demineralized enamel adjacent to the GIC containing CPP-ACP was significantly less in comparison with the conventional GIC.[5] Zhao et al. [43] tested the remineralizing effect of CPP-ACP-modified GIC and conventional GIC. Their results suggested that the CPP-ACP-modified GIC is more efficient in promoting dentin remineralization than conventional GIC which differs from the results of the current study. They used human dentin slices which were partially demineralized and bonded to a cylindrical button of different types of GIC. The current study more closely simulated the clinical situation by preparing cavities in teeth. They used micro-computed tomography to investigate lesion depth. We used both micro-hardness and EDX tests to assess the remineralizing effect. The current study found no significant difference between Fuji VII EP and Equia Forte Fil in resisting demineralization. These results might be attributed to the same or higher amount of fluoride released from Equia Forte Fil compared with the Fuji VII-EP. A study reported that Equia Forte Fil showed higher release of fluoride after 4 weeks in comparison to its predecessor GIC.[11]

The SEM/EDX analysis was conducted to evaluate changes in mineral content at the material-enamel junction. The results of the EDX test in the present study indicated the remineralizing effect of glass ionomer and glass ionomer-containing restorative materials. While a significant difference was found between the Coated Equia group and the other three groups, no significant differences were found between the first levels of Coated Equia and Fuji VII EP groups. Zhao et al.[44] showed different outcomes in their study results as they found a significant difference in Ca and P levels between conventional and CPP/ACP-modified GICs. This may be related to the analysis where they undertook SEM/EDX analysis on the dentin, while the current study used enamel. In addition, the current study used a 'hybrid' GIC instead of a conventional GIC. It was possible to observe a remineralized layer along with the restoration in the polarized light microscopy images in the current study. Zalizniak et al.[45] compared the ion release of conventional and CPP/ACP-modified GIC. Higher calcium ion release from Fuji VII EP (CPP/ACP-modified) was observed under different acidic conditions. The more important aspect of the ion release is the ion uptake by tooth structure, hence the reason for undertaking the elemental analysis of the tooth structure which is a more reliable test. According to the SEM results, incorporation of CPP/ACP into the GIC did not affect the adhesion to the tooth structure which is in agreement with the studies by Koizumi et al.[46] and Zhao et al.[43]

In the present study, the Coated Equia system (Equia Forte Fil+ Equia coat) was able to resist demineralization to adjacent tooth structure in comparison to the other materials tested. These results seem to show there is a protective effect from the Equia Coat against any acid attack which is accompanied by the additional remineralizing effect of GIC. These results agree with Gurgan et al. [47] who showed a good clinical performance of the Equia System over a four-year observation period. Brzovic-Rajic et al.[48] reported that despite the nanofilled coating that might suppress the amount of fluoride released from the GIC, the released amount is still enough to afford some protection from the acid attack from caries, especially considering the beneficial effects of the coating on the mechanical properties of the GIC.[48] The nanofilled coating has also been shown to exhibit significant resistance to wear after a 1 and 2 year follow-up period in comparison to uncoated glass ionomer restorations.[20] At a 3-year follow-up, there was still a difference in wear between coated and uncoated groups although it was not statistically different. This may be related to a lack of sensitivity in the clinical assessment of wear.[20] The results of a recent study indicated that the resin coating protects the glass ionomer materials from wear up to 20,000 cycles.[49] The nanofilled resin coating has been shown to increase the fracture strength of the GIC as well as provide a seal of the GIC to fill in surface porosities and cracks.[21] The Equia System showed superior mechanical properties than the previous generations of GIC materials, so it could be useful in selective clinical cases.

Dionysopoulos et al.[50] evaluated the fluoride release and recharge of several dental adhesives, where Fluorobond II exhibited the greatest fluoride re-release. This might be attributed to it also containing PRG fillers, and the influence of the hydrogel layer around the glass filler particles.[50] The fluoride re-release from the Giomer after recharge with NaF was comparable to the long-term fluoride release from Fuji IX Extra and better than the other two types of fluoride-containing composites.[32] Naoum et al.[32] suggested that the ability of Giomer to maintain fluoride release and fluoride recharge capability raises the possibility to reduce caries initiation on adjacent tooth surfaces compared with composites that do not contain fluoride-containing components. However, fluoride release by Fuji IX Extra was significantly greater than that of the three analyzed composites in their study. [32] In the present study, we used the combination of Giomer with FL Bond II which showed comparable results to uncoated GIC.

Conclusion and recommendation:

According to the present study, all tested materials showed some ability to resist demineralization at the restoration margins, whether enamel or dentine. Interestingly, the coated

GIC restoration showed better outcomes compared with the other tested materials. This perhaps raised the point of having a semi-permeable protective coating which allows ion movement as one means to protect a restoration margin from demineralization as well as enable remineralization. Further clinical studies on high caries risk patients are needed to confirm the findings of the present study as well as establish better restorative protocols for this group.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Table 1: Materials included in this study

Material	Composition	Manufacturer	Lot number
Cavity conditioner	20% polyacrylic acid, 3% aluminium chloride hexahydrate	GC Corp, Tokyo, Japan	1506191

Equia Forte Fil	Powder: 95%strontium fluoro alumino-silicate glass, 5% polyacrylic acid Liquid: 40% aqueous polyacrylic acid.	GC Corp, Tokyo, Japan	1608261
Fuji VII EP	Liquid: distilled water 45–55%, polyacrylicacid35–45%, poly basic carboxylic acid10-15% Powder: fluoro-alumino-silicate glass 95–99%, CPP–ACP 3%	GC Corp, Tokyo, Japan	1602191
Beautifil II	Bis-GMA, TEGDMA, S-PRG filler, multifunctional glass filler:83.3%(w/w) 0.01-4 μm	Shofu Inc., Kyoto, Japan	061757
Fl Bond II	Primer: water, Ethanol, carboxylic acid monomer, phosphoric acid monomer and initiator Bond: S-PRG filler based on fluoro alumino-silicate glass, UDMA, TEGDMA 2-HEMA, initiator	Shofu Inc., Kyoto, Japan	041501
Equia Forte Coat LC	50% Methyl methacrylate, 10-15% colloida silica, 0.09% camphorquinone, 30-40% urethane methacrylate, 1-5% phosphori ester monomer	GC Corp, Tokyo, Japan	1503121

"Coated Equia group" was restored with Equia Forte Fil then coated by Equia Forte coat.
"Beautifil II" is a second generation of giomer.

Table 2: Results of Microhardness Test (Knoop hardness, MPa)

	Enamel					Dentin				
	Level 1 (100 µm)	Level 2 (250 µm)	Level 3 (400 µm)	Level 4 (550 µm)	Mean microhardness values of Enamel	Level 1 (100 µm)	Level 2 (250 µm)	Level 3 (400 µm)	Level 4 (550 µm)	Mean microhardness values of Dentin
Coated Equia	299.6±20.9	293.9±36.9	278.6±38.6	304.9±25.6	294.27±31.47 [a]	45.2±12.9	42.2±11.9	46.4±10.4	47.5±10.2	45.32±11.04 [a]
Equia	272.3±23.2	252.9±22.0	279.5±22.5	281.4±25.4	271.51±25.37 [b]	36.8±7.3	36.5±12.3	36.8±9.2	39.0±9.0	37.26±9.2 [b]
Fuji VII EP	264.0±20.8	272.5±35.4	283.3±32.3	294.3±37.7	278.53±32.74 [ab]	30.4±8.8	32.2±6.0	30.3±5.4	31.5±5.7	31.01±6.3 [b]
Beautifil II	257.4±59.6	243.2±25.6	248.4±34.9	258.7±34.0	251.93±39.11 [c]	34.7±10.5	31.5±10.3	33.4±11.2	34.6±12.0	33.56±10.5 [b]

Data are expressed as mean ± SD. Different superscripts indicate a significant difference between different materials. Number of samples =8 in all groups. No significant difference between different levels of the same group was found ($p>0.05$).

Table 3: Results of EDX test comparing the Ca, P, and Ca/P ratio between 4 groups at different levels in enamel.

	Material	1st Level (50µm)	2nd Level (150µm)
Calcium weight	**Coated Equia**	0.31±0.01[a]	0.27±0.02[b]
	Equia	0.30±0.01[ab]	0.24±0.04[c]
	Fuji VII EP	0.31±0.02[a]	0.24±0.03[c]
	Beautifil II	0.30±0.01[ab]	0.24±0.03[c]
Phosphate weight	**Coated Equia**	0.16±0.01[a]	0.14±0.01[a]
	Equia	0.15±0.003[a]	0.12±0.02[b]
	Fuji VII EP	0.15±0.01[a]	0.12±0.02[b]
	Beautifil II	0.15±0.01[a]	0.12±0.02[b]
Ca/P Ratio	**Coated Equia**	2.04±0.15	1.90±0.08
	Equia	2.01±0.07	1.95±0.06
	Fuji VII EP	2.00±.06	1.91±0.06
	Beautifil II	2.00±0.05	1.98±0.03

Data are expressed as mean ± SD. Different superscripts indicate a significant difference between different materials. Number of samples =8 in all groups.

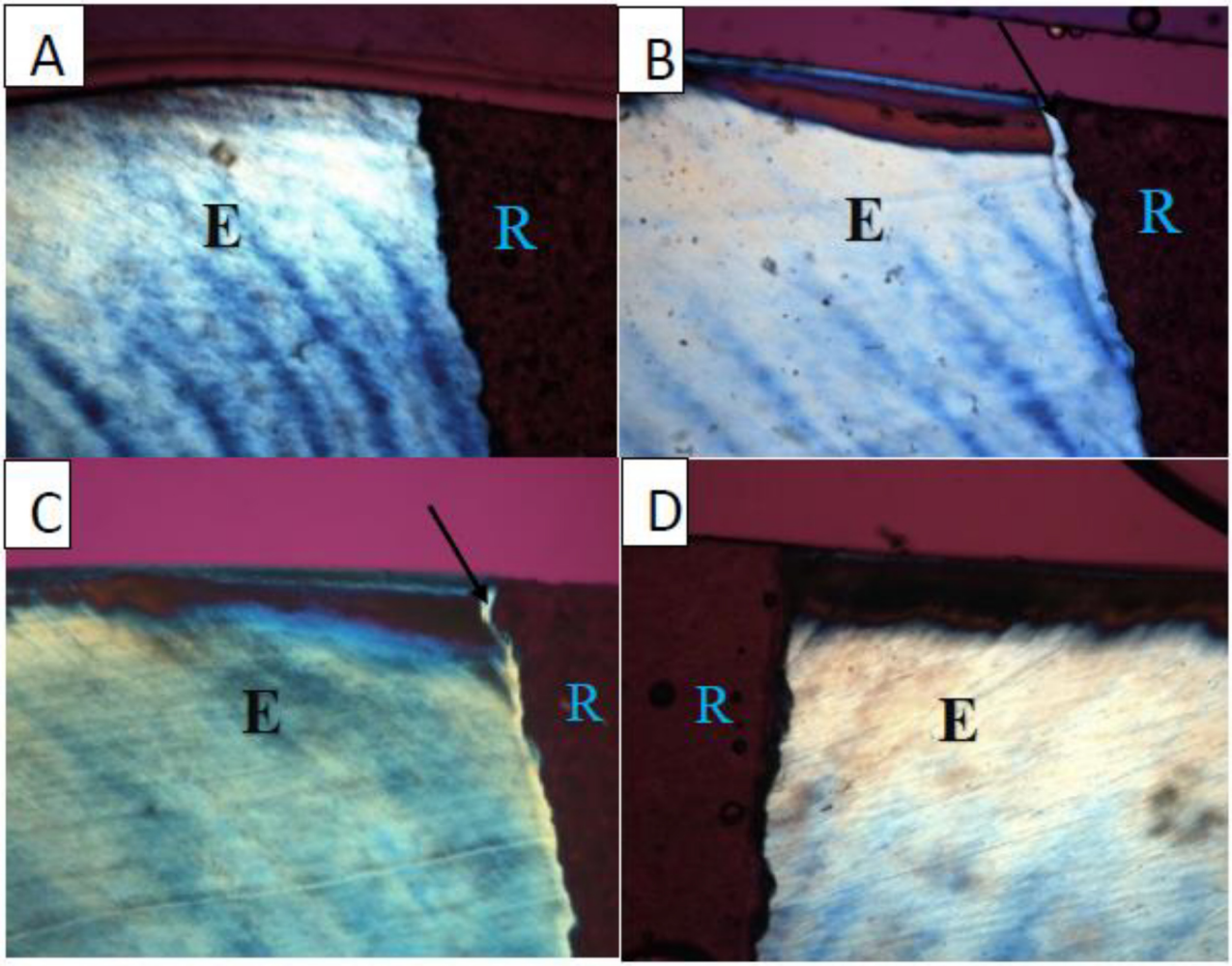
Fig. 1 Polarized light micrography showing the remineralizing effect of tested materials on Enamel margins after pH cycling (the arrow points to the remineralized area) Coated Equia group (A) showed the highest level of remineralization in comparison to Equia Fortefil group (B), Fuji VII EP group (C) and Beautifil II group (D). "R: restoration; E: enamel"

Fig. 2 Polarized light micrography showing the remineralizing effect of tested materials on dentin margins after pH cycling. Coated Equia group (A) showed protection of the dentin in comparison with Equia Fortefil (non-coated) (B), Fuji VII EP (C) and Beautifil II (D). "R: restoration; D: dentin"

Fig. 3 Polarized light micrography showing the same pattern of results in Coated Equia group with the coating layer in place (A) and the absence of coat layer accidentally removed during specimen preparation (B). "R: restoration; E: enamel"

Fig. 4 Polarized light micrography showing the same pattern of results in Coated Equia group with the coating layer in place (A) and the absence of coat layer (B), even here it can be noted there is minimal demineralization. "R: restoration; D: dentin"

Fig. 5 SEM micrographs showing acid-base resistant layer (ABR) at the interface between Equia Forte Fil (A), Fuji VII EP (B), and a hybrid layer for Fluoro Bond/Beautifil II (C) and the dentin. "D: dentin"



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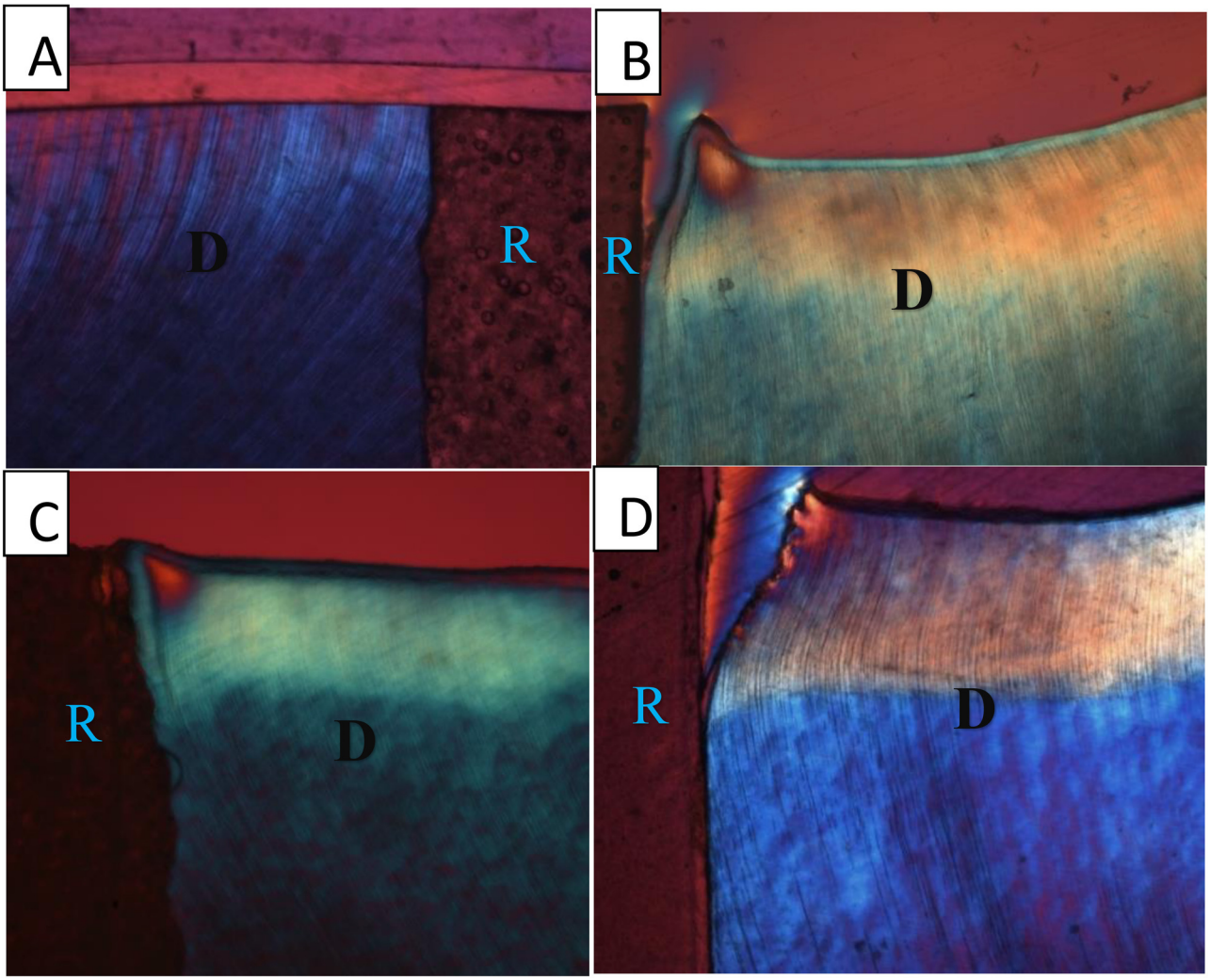

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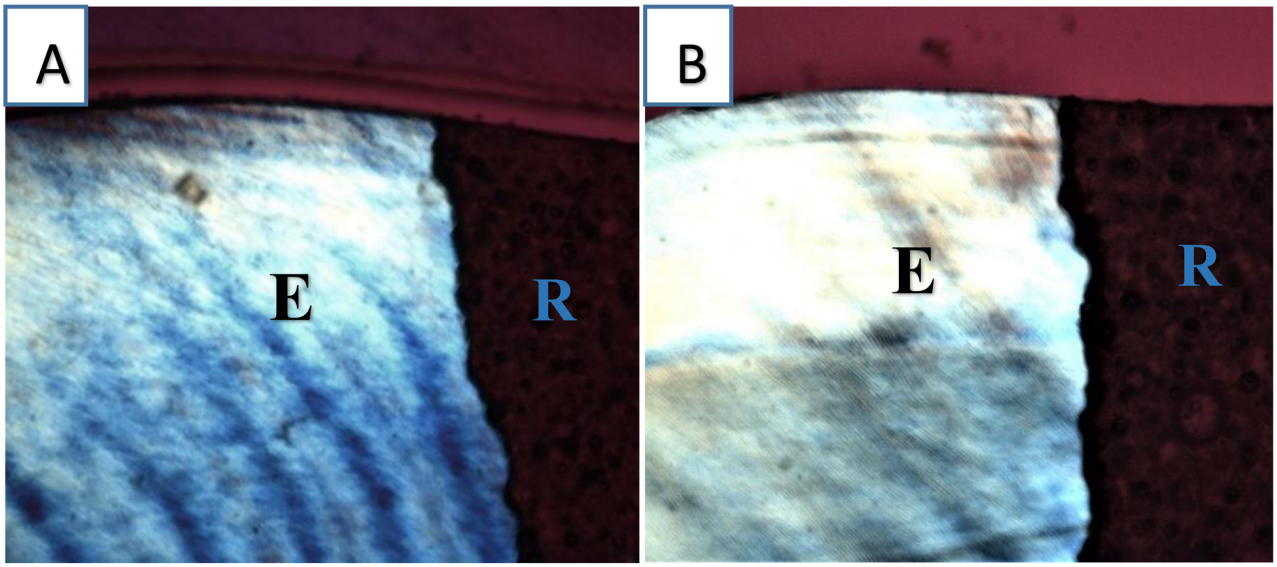

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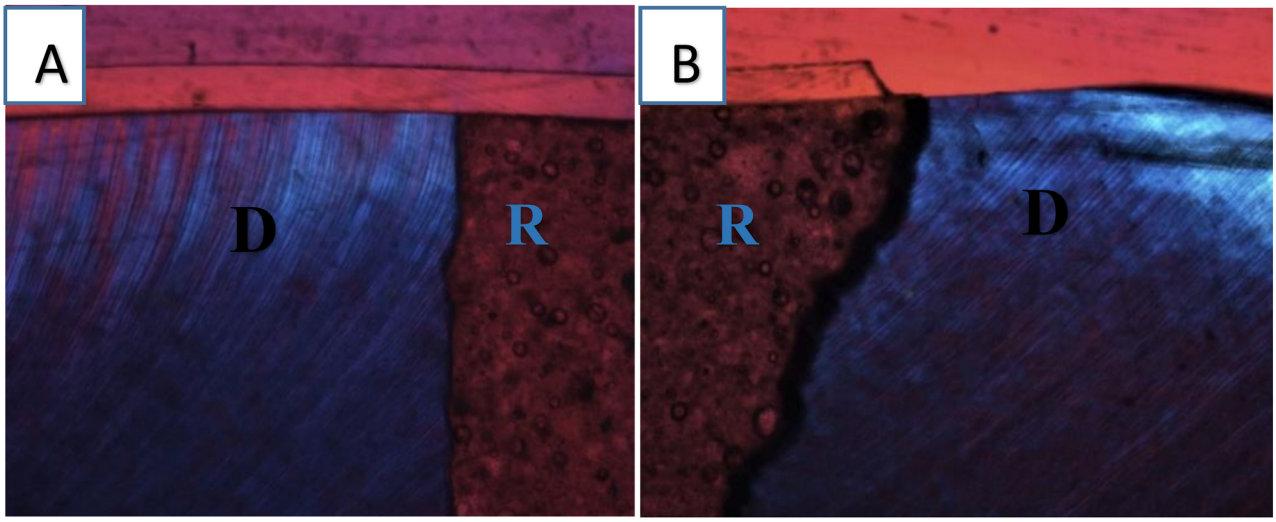

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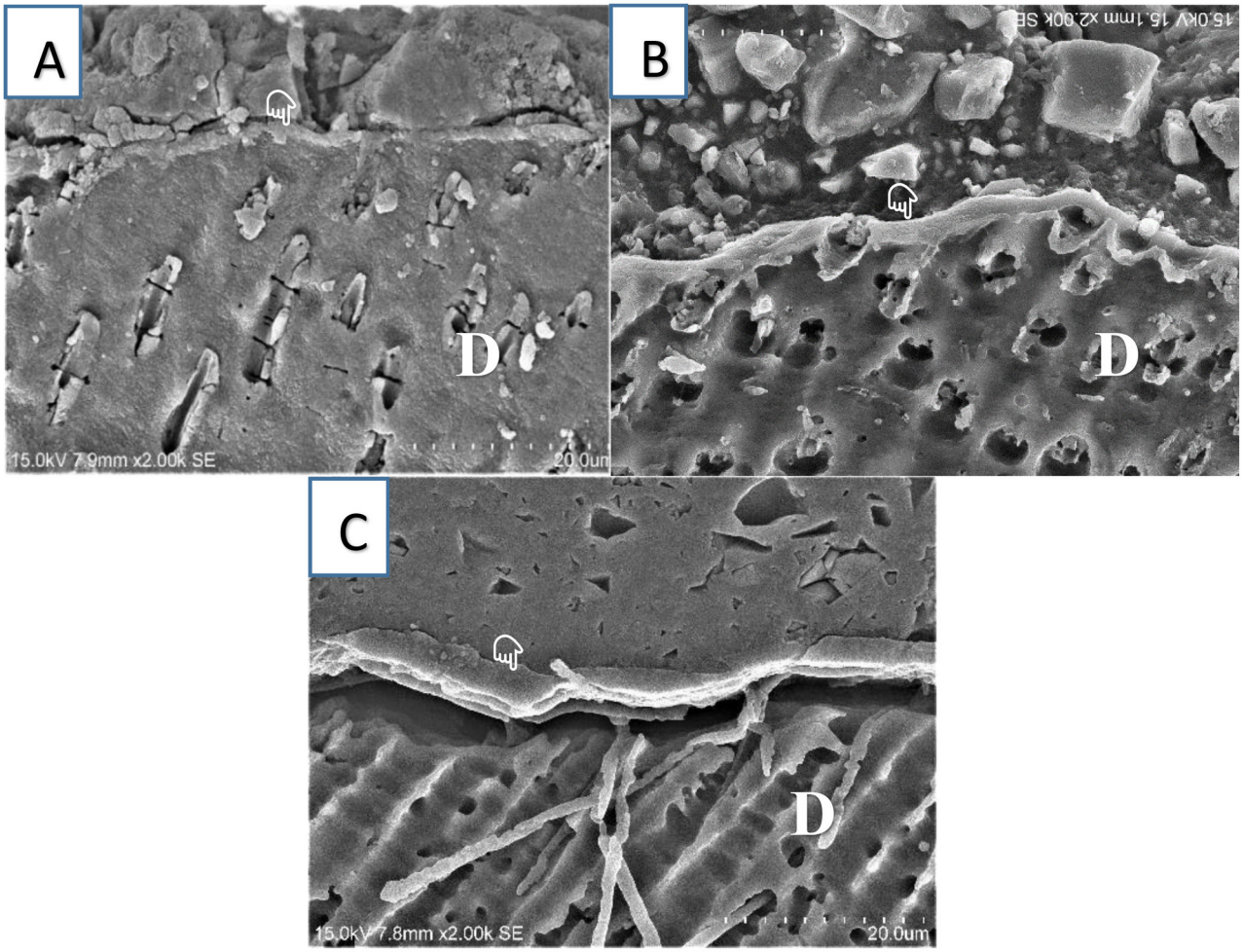

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