

## ELEMENTAL AND MICROMORPHOLOGICAL ANALYSIS OF NEW ALKASITE BASED RESTORATIVE MATERIAL/TOOTH INTERFACE

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### **ABSTRACT**

**Objectives:** To test if the alkasite-based composite could encourage the accumulation of calcium and phosphorus at tooth/restoration interface and to assess its adhesion to tooth structure using scanning electron microscope (SEM) after PH cycling.

**Method:** Cervical cavities were prepared on the buccal surfaces of 60 molars for the following restoration groups: *group 1*, Samples were kept moist without any further treatment; *group 2*, samples were kept in demineralizing solution; *group 3*, new alkasite based composite (Cention -N); and *group 4*, Cention -N with bonding agent ; *group 5*, A resin-modified glass ionomer restorative material (active bioactive composite); *group 6*, high-viscosity conventional glass ionomer restorative material (Equia Forte). The restored teeth were pH cycled for 30 days. Each tooth was buccolingually sectioned into two halves. One half was subjected to elemental analysis under low vacuum at 1000× magnification using Energy Dispersive X-Ray (EDX). The recorded values represented the average calcium (Ca) and phosphorus(P) weight percentages. The other half was sputter coated and micromorphological analysis of restoration/Tooth interface was done using SEM (JEOL, JSM 6510 IV, Japan) at 15.0 kV. Micrographs at magnifications of 2000×were captured.

**Results;** after restoration with Cention -N, Activa bioactive composite, and Equia Forte the Ca levels were significantly increased. In contrast to restorations with Cention-N with bonding agents Ca levels were insignificantly increased. P levels showed slight insignificant increase after restoration with all tested restorative materials.

**Conclusion:** The tested restorative materials showed promising results regarding remineralization.

**KEY WORDS:** Alkasite based composite, SEM-EDX, resin modified glass ionomer, conventional glass ionomer.

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## INTRODUCTION

Secondary caries surrounding an existing restoration was reported to be responsible for more than 25% of direct restoration replacement, according to Wilson et al<sup>1</sup>. Ion-releasing restorative materials are a category of restorative materials that releases ions like fluoride (F), calcium (Ca), and phosphorus (P), which can help with enamel remineralization and secondary caries reduction<sup>2</sup>. These ions can promote the formation of apatite-like substances at the tooth-restoration interface, increasing tooth resistance to acids and enhance the clinical performance of dental restorations. Furthermore, some materials can react to a drop in oral pH by releasing hydroxyl ions, which neutralize the acids generated from cariogenic bacteria and increasing the pH of the environment, preventing tooth demineralization and enhancing remineralization<sup>3</sup>. Glass ionomers are one of the first fluorides-releasing esthetic restorative materials, following silicate cements.

Glass-ionomer cements (GIC) are water-based restorative materials that release fluorides, which aid in the prevention of dental caries by increasing remineralization, reducing demineralization, and decreasing plaque formation<sup>4-6</sup>. They have the benefit over other restorative materials in that they can be inserted into cavities directly without bonding agent. Glass ionomer cement's adhesive properties and fluoride release help to lower the chance of recurrent caries in both filled teeth and adjacent tooth's enamel surfaces<sup>7,8</sup>. GICs have number of disadvantages, including moisture sensitivity and insufficient strength<sup>9</sup>, despite their proved advantages such as; bonding to tooth structure, fluoride release<sup>10</sup>, coefficient of thermal expansion and contraction close to tooth structure<sup>11</sup>, and biocompatibility<sup>12</sup>. Several GIC modifications have been introduced to dental market in an attempt to address these disadvantages.

Water soluble resin was used to modify traditional glass ionomer cement. These resins modified glass ionomer cements (RMGICs) had higher mechanical

properties, adhesion, and aesthetics, were less difficult to apply with less moisture sensitivity<sup>13</sup>. They were shown to have the same potential for fluoride release as conventional ionomer cements in most cases.<sup>14</sup> The Activa bioactive composite (ABC) is a RMGIC that may release a variety of ions, including Ca, P, and F. It's marketed as a bioactive material because it's said to produce apatite in the marginal gaps and release more fluoride than glass ionomer cements.

Equia Forte is a high viscosity GIC and a composite resin coating. During the early setting process, the resin covering protects GIC from water contamination and close surface fissures and porosities. As a result, it improves wear resistance and toughness, as well as translucency and marginal seal<sup>15</sup>.

A novel metal-free aesthetic alkasite restorative material (Cention N, Ivoclar Vivadent, Liechtenstein) has been introduced, which claimed to release significant levels of fluoride ions comparable to conventional GICs, as well as hydroxyl and calcium ions due to its patented alkaline filler<sup>16</sup>. The production of hydroxide ions from a restorative material may also help to neutralize excess acidity caused by cariogenic bacteria during acid attacks, preventing demineralization<sup>17</sup>. These factors may enhance the anticariogenic potential of Cention-N.

The morphological analysis of bonded surfaces created by adhesive systems to various dental substrates is commonly done using scanning electron microscopy (SEM). Several studies have attempted to explain bonding efficacy in terms of hybrid layer formation, integrity, and thickness, as well as resin tag production, length, and density, by assessing the quality of the bonded interface<sup>18</sup>.

Many studied investigated the ions released from Equia Forte, Activa, and Cention- N, but no available studies on the uptake of these ions by tooth structure. In this study, I aimed to determine whether Alkasite based composites can promote a buildup of major elements implicated in hydroxyapatite formation,

such as Ca and P and also to study the restoration/tooth interface with and without bonding agent. The hypothesis was that no difference in Ca and P accumulation in E between the studied material, the use of Alkaside based composites in combination with dental adhesion will enhance the bond between the material and dentin.

## MATERIALS AND METHODS

Sixty sound-extracted human molars were gathered, all of which were devoid of caries, restorations, cracks, or other defects. An ultrasonic scaler was used to clean the teeth of any soft tissues or hard calculus deposits. Selected teeth were then preserved for two weeks in 0.5% chloramine T at 4°C, then in distilled water containing thymol crystals to inhibit bacterial growth.

Box-shaped buccal cavities 3 mm long × 4 mm wide × 2 mm deep were prepared with parallel-sided diamond bur of fine grit in a high-speed hand piece with air-water cooling system along the cervical area of the crowns placing the gingival margin 1 mm occlusal to cervical line. According to the filling material employed, the teeth were then randomly divided into six experimental groups of ten teeth each.

**Group 1, (negative control group):** 10 samples were kept moist without any further treatment until the day of experimental analysis. **Group 2, (positive control group):** 10 samples were kept in demineralizing solution (50 mmol/L acetic acid, 1.5 mmol/L CaCl<sub>2</sub> and 0.9 mmol/L KH<sub>2</sub>PO<sub>4</sub> adjusted to pH 5.0 with KOH). **Group 3 (Cention -N, self-cure mode),** Two scoops of powder and two drops of resin of Cention-N were hand mixed (60 seconds) to a smooth consistency. The cavities restored with the mixed cement in bulk and left untouched for 10 minutes from the beginning of mixing according to the manufacturer's instruction. **Group 4 (Cention -N +bonding agent, light cure mode),** The cavities were etched with 37% phosphoric acid for 15-20s, then bonded with Adper Single Bond 2 (3M ESPE,

St. Paul, MN, USA) and light cured for 20s before being bulk filled with Cention-N, which was then light cured for 20 seconds. A resin-modified glass ionomer restorative material (Active bioactive composite) was used to restore **Group 5**. According to the manufacturer's directions, the material was injected into the cavity with an Activa auto mixing syringe and cured for 20 seconds after conditioning the surface with polyacrylic acid for 10 s. Equia forte, A high-viscosity conventional glass ionomer restorative material was used to restore **Group 6**. The Equia Forte capsules were mixed for 10 seconds in the capsule mixer before being withdrawn and inserted in the cavities using the GC capsule applicator.

Polishing discs were used to finish and polish all of the restored teeth. On the Equia surface, a coating of Equia Forte Coat was applied with a microbrush and light cured for 20 seconds. To allow complete polymerization of the cements, the teeth were kept in distilled water at 37°C for 24 hours. The teeth were painted with an acid-resistant varnish, leaving a window containing the restoration and 2 mm border of uncoated tooth structure.

Specimens were immersed in 10 ml of remineralizing solution (1.5 mmol/L CaCl<sub>2</sub>, 0.9 mmol/L KH<sub>2</sub>PO<sub>4</sub>, 139 mmol/L KCl, and 20 mmol/L HEPES buffer adjusted to pH 7.0 with KOH.) for 20 hours, then removed and rinsed with deionized water before being immersed in 10 ml of demineralizing solution (50 mmol/L acetic acid, 1.5 mmol/L CaCl<sub>2</sub> and 0.9 mmol/L KH<sub>2</sub>PO<sub>4</sub> adjusted to pH 5.0 with KOH) for 4 hours<sup>19</sup>. The pH cycling was done for 30 days at 37 °C, after that, specimens were carefully washed with deionized water.

Through the restoration's center, teeth were buccolingually sectioned. Silicon carbide sheets (400, 600, 1200, 2000, and 4000 grit) were used to polish the cut surfaces, final polishing was done by 1 μm diamond pastes. After that, in an ultrasonic cleaner, the specimens were cleaned in distilled water for 1 minute. The uncoated halves were subjected to EDX analysis with Oxford Aztec software to

determine the elemental composition of the enamel ( $10\mu\text{m}$  from the margins) under low vacuum at  $1000\times$ . The recorded values represented the average mineral weight percentages. The bonded interfaces of the other halves were subjected to an acid–base challenge with 10% ortho-phosphoric acid for 10 seconds before being rinsed with distilled water and 5% sodium hypochlorite solution for 5 minutes, respectively. Then were sputter coated with a platinum and palladium alloy and micromorphological analysis of restoration/tooth interface was done using scanning electron microscope (JEOL, JSM 6510 IV, Japan) at 15.0 kV. Micrographs at magnifications of  $2000\times$  were captured.

#### Statistical analysis and data interpretation:

The results were fed into a computer and evaluated using IBM SPSS Corp. software, which was released in 2013. IBM SPSS Statistics for Windows, version 22.0. Armonk, New York-based IBM Corporation. To describe qualitative data, number and percent were used. The Shapiro–Wilk test was used to confirm normality, and quantitative data was presented using mean and standard deviation for parametric data. The (0.05) level of

significance was used to determine the significance of the obtained results.

#### Parametric tests

One-Way ANOVA test was used to compare more than two independent groups, with the Post Hoc Tukey test being used to detect pair-wise comparisons.

## RESULTS

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

Table 2 represents the weight percentages of Ca and P levels on the enamel next to restorative margins ( $10\mu\text{m}$ ). Study results showed that demineralization altered Ca and P levels significantly. But after restoration with Cention -N, Active bioactive composite, and Equia Forte the Ca levels were significantly increased. In contrast to samples restored with Cention-N with bonding agents Ca levels were insignificantly increased. P levels showed slight insignificant increase after restoration with all tested restorative materials.

TABLE (1): Materials used in the study.

Product	Composition	Manufacturer	Lot number
<b>Cention -N</b> (alkasite based composite)	<i>Liquid:</i> Dimethacrylates, initiators, stabilizers, additives and mint flavour. <i>Powder:</i> Calcium fluoro-silicate glass, barium glass, calcium-barium-aluminium fluoro-silicate glass, iso-fillers, ytterbium trifluoride, initiators and pigments.	Ivoclar vivadent AG FL-9494 Schaan/ Liechtenstein	Z00547
<b>Activa Bioactive</b> (RMGI)	Blend of diurethane and other methacrylates with modified polyacrylic acid (44.6%), Amorphous silica (6.7%), Sodium fluoride (0.75%).	Pulpdent Corporation, Watertown, MA US	160314
<b>Equia Forte</b> (conventional GIC)	<i>Powder:</i> Fluoro-alumino-silicate glass, Polyacrylic acid powder, Pigment <i>Liquid:</i> Polyacrylic acid, Distilled water, Polybasic carboxylic acid.	GC Dental, Japan	Tokyo, 150213B

TABLE (2): Means and standard deviations of Ca and P levels between studied groups

	-VE Control n=10	+VE Control n=10	Cention-N n=10	Cention-N+ bonding agent n=10	Active bioactive n=10	Equia Forte n=10	Test of significance
Ca	33.11±4.56	11.59±2.39 <sup>a</sup>	16.73±2.79 <sup>bdc</sup>	13.67±4.38 <sup>abc</sup>	15.14±3.49 <sup>cdf</sup>	16.97±2.87 <sup>ef</sup>	F=48.58 P<0.001*
P	16.12±3.04	8.15±2.81 <sup>acd</sup>	9.27±2.48 <sup>abeh</sup>	8.17±2.57 <sup>befi</sup>	9.36±3.32 <sup>defk</sup>	10.15±2.72 <sup>ghik</sup>	F=11.16 P<0.001*

*F*;One Way ANOVA test , similar superscripted letters denote non- significant difference between groups by Post Hoc Tukey test \*statistically significant

**Micromorphological Analysis Using Scanning Electron Microscope (SEM):**

The scanning electron micrographs in Figs. 1 to 4 show the SEM observations of the restoration/dentine interfaces. *Cention-N/dentin interface* showed good adaptation between the restoration and tooth substrates but no signs of resin-interdiffusion. Micromechanical locking was more limited to surface roughness and irregularities. *Cention-N*

*with bonding agent*; the interface sealed as an acid-resistant, resin-dentin interdiffusion zone. The hybrid layer was well defined and resin tags were observed. *The Activa Bioactive/dentin interface* revealed cement matrix penetration into dentinal tubules and the development of a hybrid-like layer. *The Equia Forte/dentin interface* demonstrated an interdiffusion zone or ‘ion exchange layer’ between the cement matrix and the dentine.

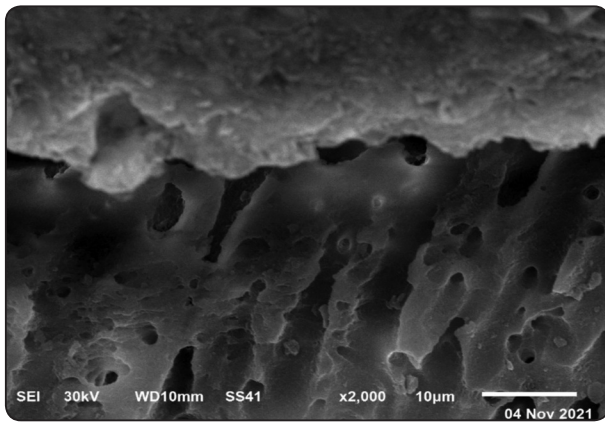


Fig. (1): Cention-N/dentin interface

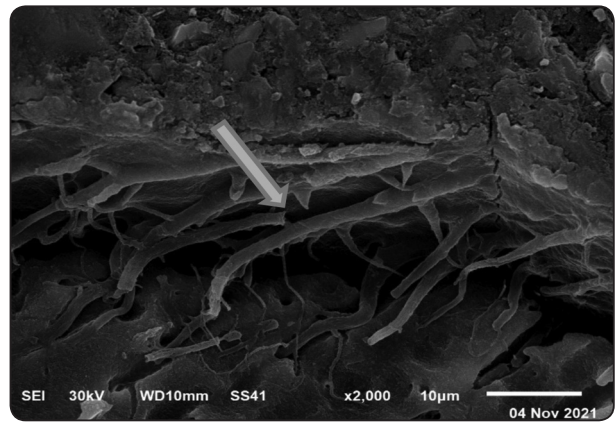


Fig. (2): Cention-N with bonding agent; hybrid layer (arrow)

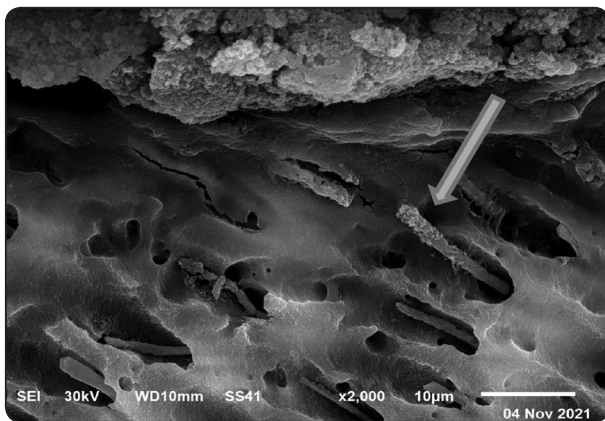


Fig. (3): Activa Bioactive/dentin interface Resin tags and signs of remineralization(arrows)

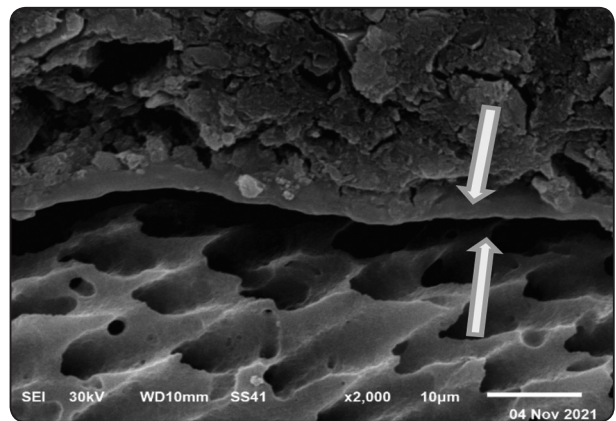


Fig. (4): The Equia Forte/dentin interface; ion exchange layer (arrows)

## DISCUSSION

The samples were subjected to long-standing pH cycle replicating an acidic-to-neutral microenvironment to imitate a demineralization–remineralization process that occurs naturally. It mimics the behavior of a dynamic process, because dental caries is a process of alternating demineralization and remineralization that is a direct result of conditions that keep the mouth's pH at a critical level<sup>20</sup>. EDX elemental analysis is a conservative and simple method to evaluate mineral content in small areas of tooth structure<sup>21</sup>. No studies have been carried out on the interactions that take place when the Cention N is brought into contact with the tooth structure.

The EDX analysis results revealed an increase in Ca and P levels in the enamel margins next to all the tested restorative materials indicating their ability to remineralize the enamel layer near the restoration but with no significant difference between them. The increase in Ca ions was significant in contrast to the increase in P ions, this finding could be a result of the filler content of these materials. For glass ionomer (Equia Forte), the setting reaction also plays a role where a layer of calcium salts and aluminium polyacrylate formed by chelation of the tooth surface calcium ions by the carboxylic radicals in the polyacrylic acid. The development of an intermediate layer on the interface, which is formed by elements from both the cement and the tooth structure, as well as the intertubular infiltration of cement calcium ions into the dentin, was shown by Fourier Transform Infrared Spectroscopy (FTIR)<sup>22,23</sup>. X-ray tests revealed ionic exchanges between carboxylate ions in the polyalkenoic acid and calcium in the tooth<sup>24</sup>.

The fillers of Cention-N comprise barium aluminum-silicate glass filler, ytterbium trifluoride, an isofiller, a calcium barium aluminum fluorosilicate glass filler, and a calcium fluorosilicate glass filler. According to the manufacture a 0.5- $\mu$ m thick surface layer of calcium fluoride and calcium

phosphate, has been observed, which is resistant toward washing with deionized water<sup>16</sup>. Cention-N in self-cure mode released substantially higher Ca ions than that of light-cure mode. Because of photopolymerization of the alkasite restorative material, the capacity of light cure to release Ca ions has decreased. This could be attributed to a covalently bound or less hydrophilic matrix. Also this may be attributed to the used adhesive which form a hybrid layer as confirmed by SEM image which prevents the diffusion of Ca and P ions into tooth structure<sup>16</sup>. The results are in accordance with Gupta et al who found that Cention-N (self-cure) has more fluoride ion release and alkalizing capability in acidic pH as compared to Cention-N (light-cure) and GIC<sup>25</sup>. The results are in consistent with Kasraei et al who reported Ca and p ion release from activa bioactive composite and Cention N and concluded that these bioactive materials can have a long term positive effect on tooth remineralization<sup>26</sup>. The results also in accordance with Tiskaya et al<sup>27</sup> who reported ion release from Activa Bioactive and Cention\_N which varies according to the pH of the immersion medium.

Micromorphology of the interface of (Cention-N) showed no signs of resin-interdiffusion which may be attributed to the smear layer which left plugging dentinal tubule and no adhesive resin was used, so micromechanical locking limited to surface roughness and irregularities created by the cutting instrument. In contrast to teeth restored with alkasite based composite (Cention-N) with bonding agent, the interface was sealed as an acid-resistant, resin-dentin interdiffusion zone with resin tags extending into the dentinal tubules. This may result from the use of conventional two-step adhesive system which depends on removal of smear layer, unplugging of dentinal tubules, and demineralization of dentin exposing the collagen fibrils by acid etching followed by application of adhesive resin to polymerize insitu forming the hybrid layer.

The Micromorphology structure at the interface of RMGI (activa bioactive) revealed good adaptation between the RMGICs and the underlying dentine with resin tags infiltrating the dentinal tubules. Because RMGICs have resin in its components, a unique bonding process is expected. Lin et al.<sup>28</sup> concluded that mechanical interlocking may give extra stability to the RMGICs via resin monomer penetration into the dentinal tubules. The study results were in agreement with Tanumiharja et al.<sup>29</sup> and Pereira et al.<sup>30</sup> who observed the presence of resin tags in the dentinal tubules for RMGIC and attributed that to conditioning with dentin conditioner.

The self-cured GIC (Equia Forte) interfacial micromorphology showed a good bond between the self-cured GIC and the tooth structure. This interfacial zone is known as the 'ion exchange layer' or 'hybridlike layer'.<sup>29</sup> Ionic exchange between the minerals of the tooth structure and the organic components of the GIC is the mechanism through which self-cured GICs bond to tooth structure. The first stage is a weak hydrogen bond between the prepared cavity walls and freshly mixed cement due to polar attraction. Studies have shown that the acidity of the cement acts as a self-demineralizing agent on the tooth smear layer<sup>31,32</sup>. Gradually, the hydrogen bonds are replaced by a stronger chemical bond of a polar/ionic type. The existence of cement matrix in the dentinal tubules suggests that the polyacrylic acid component of the cement was able to disrupt the smear plugs, allowing the cement to permeate the tubules<sup>33</sup>.

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