

# Electron probe microanalysis of ion exchange of selected elements between dentine and adhesive restorative materials

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## Abstract

**Background:** There have been numerous attempts to demonstrate the phenomenon of ion exchange between auto cure glass ionomer cements (GICs) and dentine. The purpose of this study was to employ an electron probe microanalysis (EPMA) technique to examine the interchange of elements between non-demineralized dentine and two types of restorative material, auto cure GICs and a resin composite.

**Methods:** Restorations of auto cure GICs (Riva Fast, Fuji IX Fast, Ketac Molar Quick and Fuji VII) and a bonded composite resin were placed in each of 10 recently extracted human third molar teeth. After two weeks the restorations were sectioned and prepared for EPMA. Percentage weights of calcium, phosphorus aluminum, strontium and fluoride were calculated in the restorations 200µm from the restorative interface and 200µm into the dentine at 5µm intervals.

**Results:** There was evidence of calcium and phosphorus in all five auto cure GICs to a depth of 50µm. Aluminum and strontium ions were also present in dentine except subjacent to Ketac Molar restorations. There was evidence of element transfer into composite resin and resin-bonded dentine.

**Conclusions:** The findings of this paper support the concept of ion exchange as a bonding mechanism between auto cure GIC and dentine. Element penetration into tooth structure and GIC exceeded beyond the "ion exchange layer" observed in scanning electron microscopy studies. Penetration of calcium and phosphorus into composite resin from dentine likely occurred as a result of the self-etching process dissolving calcium and phosphorus and incorporating these elements into the hybrid layer. The presence of Al and Sr ions in dentine were likely to be associated with resin tags extending into the dentine.

**Key words:** Dentine, auto cure glass ionomer cement, ion exchange.

**Abbreviations and acronyms:** EPMA = electron probe microanalysis; GIC = glass ionomer cement; SEM = scanning electron microscopy.

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## INTRODUCTION

Since Wilson *et al.*<sup>1</sup> postulated the bonding mechanism of auto cure glass ionomer cement (GIC) to tooth structure there have been a number of attempts using different mechanisms to verify this bond. Watson *et al.*<sup>2,3</sup> used fluorescent dyes in GICs to demonstrate the possibility of ion exchange, Lin *et al.*<sup>4</sup> looked at the bond using secondary ion mass spectrometry and Ngo *et al.*<sup>6</sup> used a cryo scanning electron microscopy to demonstrate the existence of an interaction zone at the dentine restorative interface that resisted acid etching suggesting the existence of an ion-enriched layer. Yoshida *et al.*<sup>5</sup> confirmed ionic bonding between the carboxyl ions from the cement and calcium and phosphorus ions within the tooth using X-ray photon spectrometry. Using an electron probe microanalysis (EPMA)<sup>7</sup> technique Ngo<sup>8</sup> was able to demonstrate the transfer of strontium and fluoride ions from auto cure GICs into demineralized dentine. This result has been confirmed by other studies.<sup>9,10</sup>

The purpose of the study was to employ a similar protocol used by Ngo<sup>8</sup> to examine the transfer of specific elements (calcium, strontium, aluminum, phosphorus and fluoride)<sup>11</sup> between auto cure GICs and non-demineralized dentine and between composite resin restorative materials and non-demineralized dentine.

## MATERIALS AND METHODS

Ten recently extracted human third molar teeth that had been stored in 0.5% chloramine were cut horizontally into sections about 5mm thick so as to incorporate the cemento-enamel junction. Teeth were

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collected within the guidelines set by the Committee for the Ethics of Human Experimentation at The University of Adelaide. Five cavities of about 2mm diameter and 2.5mm deep were prepared at the enamel dentine interface so that the bases of each preparation were in dentine.

### Restoration placement prior to experimentation

Each cavity preparation was restored as follows: (1) The cavity was treated with SE Bond (Kuraray, Tokyo, Japan) according to the manufacturer's instructions and a composite resin (Ice, Southern Dental Industries Melbourne, Australia) was placed into the preparation and photo cured for 10 seconds; (2) The cavity was conditioned with GC conditioner (GC Corporation, Tokyo, Japan) for 10 seconds, washed and dried with oil-free air. Into this preparation a GIC (Riva Fast, SDI) was placed and allowed to set; (3) The cavity was conditioned with GC conditioner for 10 seconds, washed and dried with oil-free air. Into this preparation a GIC (Fuji IX Fast, GC) was placed and allowed to set; (4) The cavity was conditioned with GC conditioner for 10 seconds, washed and dried with oil-free air. Into this preparation a GIC (Ketac Molar Quick, 3M ESPE, Minneapolis, USA) was placed and allowed to set; (5) The cavity was conditioned with GC conditioner for 10 seconds, washed and dried with oil-free air. Into this preparation a GIC (Fuji VII, GC) was placed and allowed to set.

Each restoration was then finished flush to the tooth surface using fine Emery discs and polished using fine Softflex discs (3M) and stored in an aqueous solution for two weeks.

### Specimen preparation for electron probe microanalysis

After two weeks the samples were sectioned to expose a cross-section of the dentine restoration interface at the cavity base. Each section was then placed in a fixing solution containing 1.25% glutaraldehyde, 4% sucrose, 4% paraformaldehyde in PBS at pH 7.2 (Adelaide Microscopy, Adelaide, Australia) for 12 hours, placed into a washing buffer solution, containing PBS, 4% sucrose (Adelaide Microscopy) for 1.5 hours with changes every 30 minutes. Samples were then dehydrated with ascending grades of ethanols (25% ethanol for 20 minutes, 50% ethanol for 20 minutes, 75% ethanol for 20 minutes, 95% ethanol for 30 minutes, 100% ethanol for one hour).

Dehydrated specimens were placed face down on the base of cylindrical mounting blocks into which epoxy resin 100:25 (Epoxy resin LC 191:Epoxy hardener HY 956) was poured under vacuum and left to set for 24 hours at room temperature. After setting, the specimens were polished using an Abramin polishing machine (Struers, Denmark). The upper and lower surfaces of the mounting blocks were made parallel to each other using a leveling device and the samples

mounted on a polisher. The surfaces in which the specimens were embedded were polished using a p80 grit silicone carbide disc (Struers, Denmark) at 150 revolutions per minute (rpm), lubricated with water for 30 seconds under a load of 100N. The specimen containing surfaces were then polished using p500 grit silicon carbide discs at 150rpm, lubricated with water under a load of 100N to grind away the surface resin.

Once the specimen containing surfaces were flat, the surfaces were further polished using diamond polishing discs (Struers) with diamond paste (Kemet, UK). First the surface was polished with 15 $\mu$ m diamond paste on a 15 $\mu$ m diamond polishing disc at 150rpm for five minutes at 200N, lubricated with DP-lubricant Green (Struers). After that the surfaces were polished with 3 $\mu$ m (Kemet) and 1 $\mu$ m (Kemet) diamond polishing discs and diamond pastes respectively. Both cycles were at 150rpm for three minutes at 200N and lubricated with DP-Lubricant Green. The surfaces were cleaned with water, air dried and viewed under a stereo microscope (Zeiss, West Germany) at 25x magnification to determine that the embedded specimens had been polished adequately.

The samples were carbon coated for EPMA investigation (CAMECA, SX51, France). Line scans were carried out on the specimens to measure the relative percentage weights of the following elements: calcium (Ca), phosphorus (P), aluminum (Al), strontium (Sr) and fluoride (F). Scans were conducted commencing 200 $\mu$ m within the restoration to the tooth restorative interface and to a depth of 200 $\mu$ m into the dentine every 5 $\mu$ m over a total distance of 400 $\mu$ m. One scan was made at each location. The scale of percentage weights were enhanced 75 $\mu$ m either side of the restorative interface to a field height of 5 per cent weight in order to observe more accurately ion transfers close to the restorative interface. Measurements were expressed as a relative percentage weight of the identified element as part of the total weight of the sample where the measurement was taken.

### RESULTS

Figure 1 shows a typical example of the scanning zone of each specimen. Figures 2–4 show the percentage weight of the elements calcium, phosphorus, aluminum, strontium and fluoride detected in the test

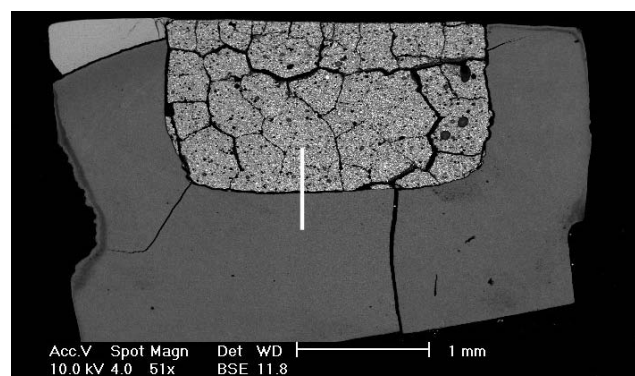


Fig 1. Vertical white lane shows typical scanning range of each sample.

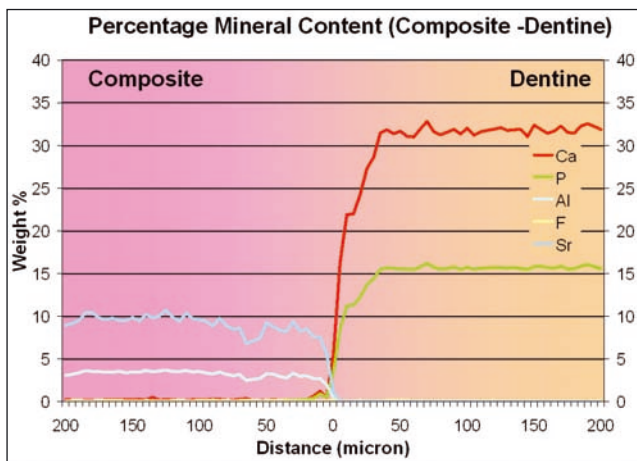


Fig 2a. Element ion transfer of calcium, phosphorus, aluminum, fluoride and strontium between composite resin and dentine. The restorative interface occurs at 0 $\mu$ m.

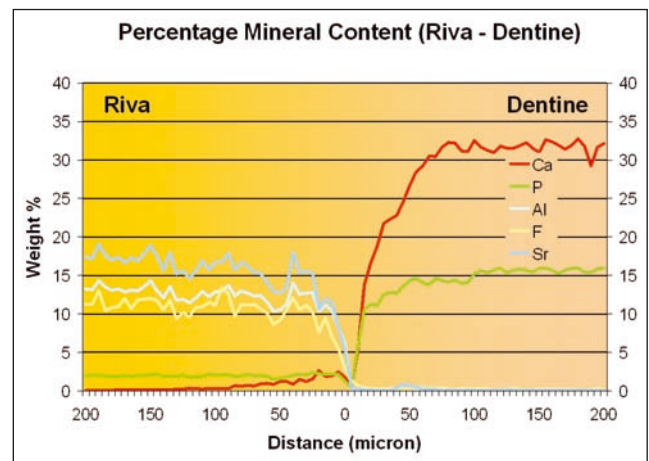


Fig 3a. Element ion exchange of calcium, phosphorus, aluminum, fluoride and strontium between Riva auto cure glass ionomer cement and dentine. The restorative interface occurs at +15 $\mu$ m.

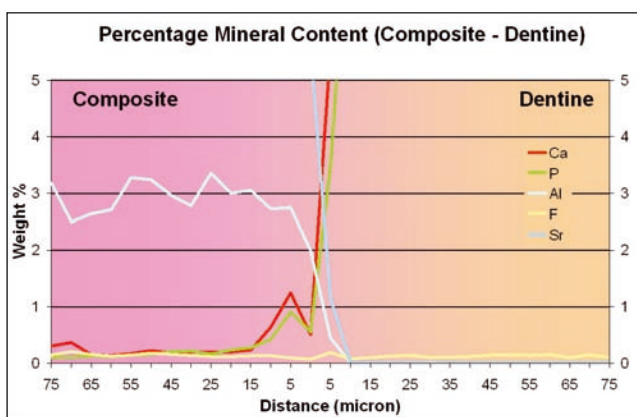


Fig 2b. Element ion transfer of calcium, phosphorus, aluminum, fluoride and strontium between composite resin and dentine enhanced to a full field of 5 per cent at the restorative interface. The restorative interface occurs at 0 $\mu$ m.

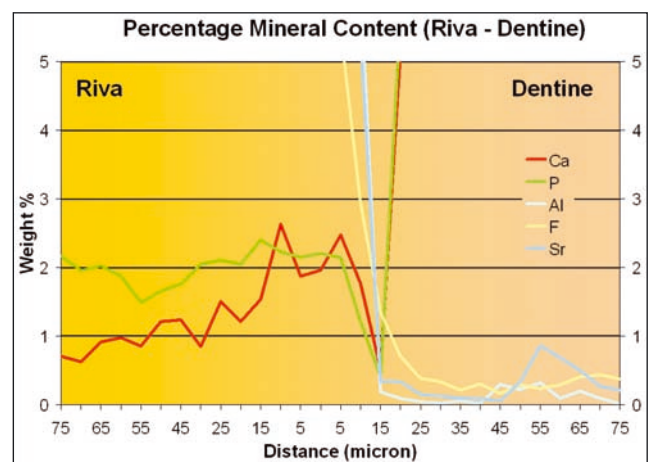


Fig 3b. Element ion exchange of calcium, phosphorus, aluminum, fluoride and strontium between Riva auto cure glass ionomer cement and dentine enhanced to a full field of 5 per cent at the restorative interface. The restorative interface occurs at +15 $\mu$ m.

restorations and in the subjacent dentine. The distribution of calcium and phosphorus in the body of the dentine in all specimens followed a similar pattern. Calcium was approximately 30 per cent by weight and phosphorus approximately 15 per cent by weight until the interface area where the levels dropped sharply. In the case of the resin composite (Figs 2a and 2b) there was a slight peak of these elements approximately 7 microns into the restoration zone. With two of the GICs (Riva and Fuji IX) detectable levels of calcium were recorded approximately 70 $\mu$ m into the restoration and detectable levels of phosphorus were seen to the limit of measurement depth.

A similar distribution to the above with respect to calcium and phosphorus were seen in the Ketac Molar specimens with the exception that there were appreciable calcium levels within the body of the restoration (approximately 10 per cent by weight).

Negligible amounts of fluoride were detected in the resin-composite specimens and in all GIC specimens the levels were between 10 and 17 per cent by weight. These levels dropped markedly at the interface area and there was some evidence of fluoride in the subjacent dentine surface zone.

Strontium was detected in the body of the resin-composite and in two of the GICs (Riva and Fuji IX). The levels were between approximately 10–17 per cent by weight. Towards the interface area the levels dropped sharply and some strontium was detected in the surface zone of the subjacent dentine. The levels of aluminum detected were lower but followed the same general distribution pattern as strontium.

## DISCUSSION

Ionic exchange between tooth structure and an adhesive restorative material requires an aqueous environment in order for ion exchange to occur.<sup>12</sup> Polyalkenoic acid from the cement is buffered by phosphate ions within the tooth and calcium and phosphate ions are dispersed from the hydroxyapatite into the unset cement. Ions are also able to disperse from the cement into the dentine resulting in an intermediate layer between the unset GIC and hydroxyapatite, referred to as the “ion exchange layer”.<sup>13</sup> Although the so-called ion exchange layer appears no more than several micrometres wide<sup>6</sup> when



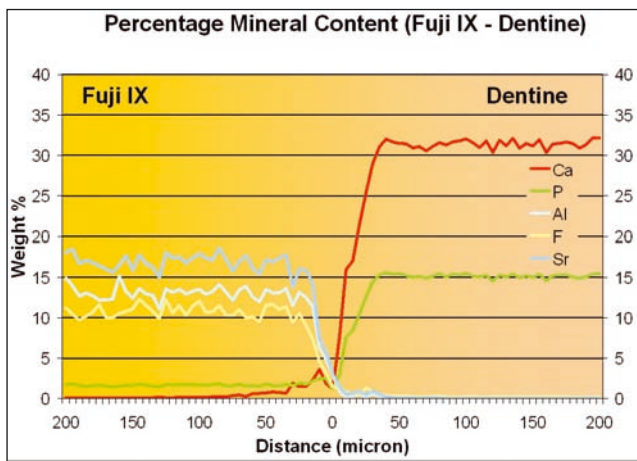


Fig 4a. Element ion exchange of calcium, phosphorus, aluminum, fluoride and strontium between Fuji IX auto cure glass ionomer cement and dentine. The restorative interface occurs at +5µm.

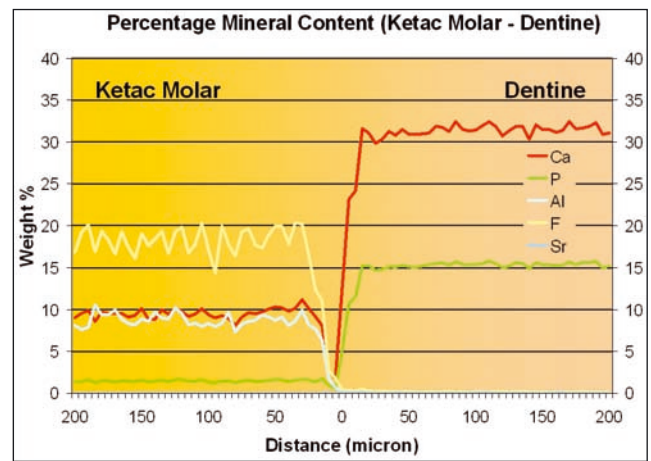


Fig 5a. Element ion exchange of calcium, phosphorus, aluminum, fluoride and strontium between Ketac Molar auto cure glass ionomer cement and dentine. The restorative interface occurs at 0µm.

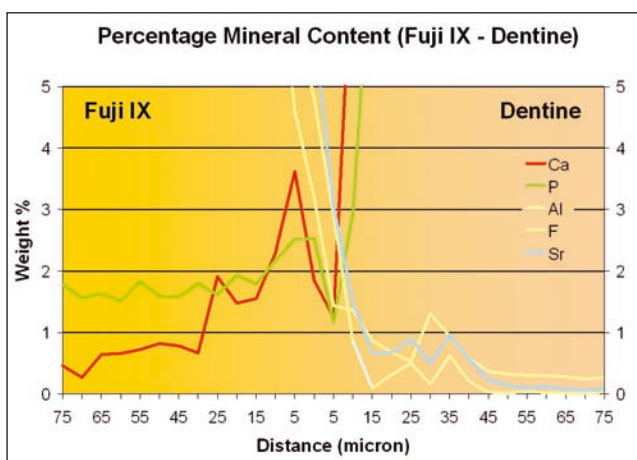


Fig 4b. Element ion exchange of calcium, phosphorus, aluminum, fluoride and strontium between Fuji IX auto cure glass ionomer cement and dentine enhanced to a full field of 5 per cent at the restorative interface. The restorative interface occurs at +5µm.

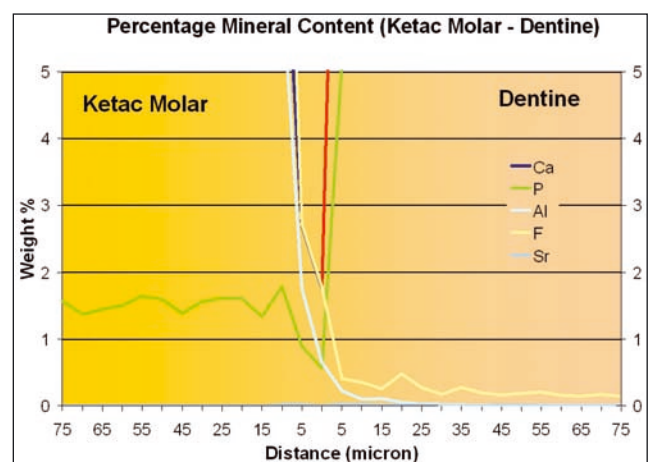


Fig 5b. Element ion exchange of calcium, phosphorus, aluminum, fluoride and strontium between Ketac Molar auto cure glass ionomer cement and dentine enhanced to a full field of 5 per cent at the restorative interface. The restorative interface occurs at 0µm.

viewed using scanning electron microscopy (SEM), the penetration of ion exchange observed using EPMA within both the dentine and GIC extend beyond 50µm either side of the restorative interface.

Elements such as silicon were not measured and it would be of interest to see how many other elements transfer between the two materials. This raises further questions as to what these characteristics may have on the bond strength and the anecdotal anticariogenic properties of GICs.<sup>14</sup>

The fluoride levels in Ketac Molar are greater than those measured in Fuji IX, Fuji VII and Riva. It is surprising that the amount of fluoride ions transferred into the dentine is similar to the other GICs measured. As Ketac Molar has a calcium-containing glass filler, element transfer is less obvious to observe. However, there is evidence of a slight spike occurring in both the calcium and phosphorus baselines close to the restorative interface that indicates calcium and phosphorus may have become incorporated from the dentine into the Ketac Molar.

Fuji VII is promoted as a high fluoride release GIC, yet the amount of fluoride released into the adjacent dentine is similar to that of Fuji IX. This suggests that although more fluoride ions are being released into the environment, there is a limit to the amount of fluoride that is capable of being deposited into dentine from auto cure GICs.

The presence of Ca and P ions embedded in the composite resin is intriguing as ion exchange can only occur in an aqueous environment. As SE Bond incorporates a self-etching primer it can be expected that a small amount of calcium and phosphorus dissolved out from the dentine surface during the etching process to become embedded into the composite resin. The extent that this may have on weakening the resin dentine bond is unknown.

The Sr and Al elements in dentine subjacent to the composite resin may have been derived from the inorganic filler fraction of the material. Studies using self-etching primers for dentine bonding on extracted human teeth have shown extensive penetration of resin

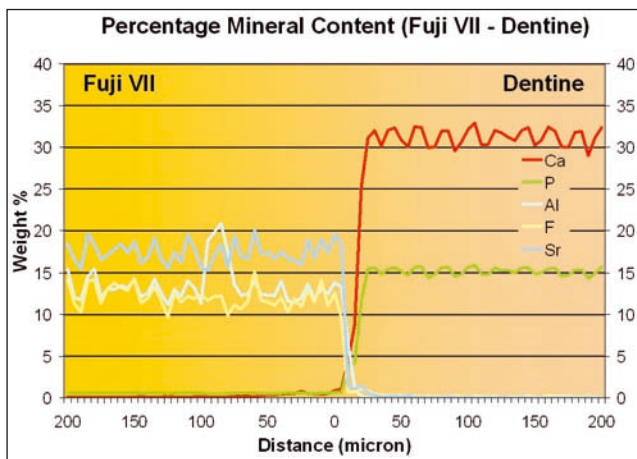


Fig 6a. Element ion exchange of calcium, phosphorus, aluminum, fluoride and strontium between Fuji VII auto cure glass ionomer cement and dentine. The restorative interface occurs at 0 $\mu$ m.

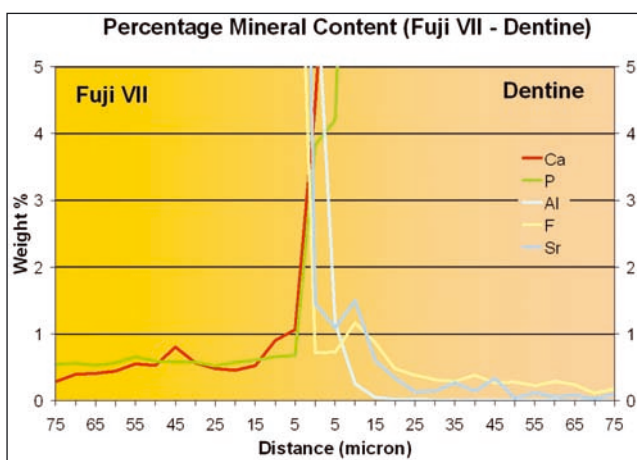


Fig 6b. Element ion exchange of calcium, phosphorus, aluminum, fluoride and strontium between Fuji VII auto cure glass ionomer cement and dentine enhanced to a full field of 5 per cent at the restorative interface. The restorative interface occurs at 0 $\mu$ m.

tags into the dentine tubules.<sup>15</sup> Whether the Sr and Al elements followed such a pathway is not known.

This pilot study supports the bank of evidence of a chemical bond between tooth structure and auto cure GICs and furthers confidence amongst dentists in the clinical application of this material.

## CONCLUSIONS

The findings of this paper support the concept of ion exchange as a bonding mechanism between auto cure GIC and dentine. Using EPMA the penetration of elements from tooth structure and GIC were found to exceed well beyond the confines of the “ion exchange layer” observed in previous SEM studies. Penetration of calcium and phosphorus into composite resin from dentine likely occurred following self-etch priming whilst the Al and Sr elements seen in the subjacent dentine may have been derived from the inorganic filler fraction of the material.

## ACKNOWLEDGEMENTS

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## DISCLOSURE

The corresponding author was associated with the development of Fuji VII and has a financial interest in this product.

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