FMUC FACULDADE DE MEDICINA
UNIVERSIDADE DE COIMBRA

Analysis of the Interface between calcium silicate-based endodontic materials and root canal dentine: *a pilot study*

Mestrado Integrado em Medicina Dentária

Olavo Guerreiro Viegas

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Orientador: Professor Doutor João Miguel Marques dos Santos

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ABSTRACT

This study evaluated by scanning electron microscopy the interface between calcium silicate-based endodontic materials (MTA and Biodentine™) and root canal dentine using three different irrigation protocols. Root canals of 66 human teeth were subjected to biomechanical preparation with ProTaper rotary instruments up to a finish file F4. The teeth were assigned into six groups according to the irrigation protocol and obturation material employed: 1A. NaOCl + MTA; 1B. NaOCl + Biodentine ™; 2A. NaOCl/NaCl/EDTA + MTA; 2B. NaOCl/NaCl/EDTA + Biodentine™; 3A. NaOCl/NaCl/CHX + MTA; 3B. NaOCl/NaCl/CHX + Biodentine™. After obturation teeth were stored for 5 days at 37ºC in a moist environment to allow the set of sealers. One section of 3mm thick was obtained from the apical third. The specimens were immersed in PBS for 5 days. After this period of time, one specimen of each group was sectioned longitudinally into two symmetrical pieces. One of the two halves - the one with the material - was processed for morphological observation, element mapping and chemical analysis along the dentine-material interface using SEM and EDS. Along the material-dentine interface, both materials formed a tag-like structure that was more evident in groups where EDTA was used for final irrigation protocol. Apatite crystal formation was visible along the interface and within the interfacial dentine in all groups. The Ca depth was constantly larger than the Si depth but the uptake of Si was more prominent in all groups for Biodentine™. Both Biodentine™ and MTA caused the uptake of Ca and Si in the adjacent root canal dentine in the presence of PBS. The dentine element uptake for Si was more prominent in Biodentine™ than in MTA.

Keywords: bioactivity, Biodentine, calcium, element uptake in dentine, mineral trioxide aggregate, silicon, scanning electron microscopy, interfaces, calcium silicate-based, endodontics, root canal filling materials.

INTRODUCTION

Mineral trioxide aggregate (MTA) is a calcium silicate-based endodontic material that has been developed by modification of Portland cement[1,2]. The MTA patent shows that it contains calcium oxide (CaO) and silicon (SiO). Several investigations have reported that the main elemental components of MTA are calcium and silica, as well as bismuth oxide[3]. It is considered the most adequate material for various treatments such as root-end filling[3,4], root canal sealer, direct pulp capping, pulpotomy, perforation repair, treatment of internal and external resorption, horizontal root fracture, apexification and apical barrier for teeth with necrotic pulps and open apexes[3-5].

The bioactivity of MTA has been attributed to its ability to produce surface apatite crystal when in contact with phosphate solutions such as phosphate-buffered saline (PBS)[6,7]. The crystalline precipitates are formed through interaction of Ca and OH ions released from set MTA[8] with phosphates and have been identified as calcium-deficient B-type carbonated apatite precipitates produced via an amorphous calcium phosphate phase[9]. Moreover, apatite crystal formation has also been demonstrated along the MTA-dentine interface and within the interfacial dentine[10-12]. These findings lead to the notion that apatite formation contributes to leakage reduction not only by filling the gap along the interface but also via dentine interactions such as intrafibrillar apatite deposition. This is supported by the finding that immersion in PBS decreases marginal leakage of MTA apical plugs[13].

Several new calcium silicate-based materials have been recently developed[14,15], aiming to improve some MTA drawbacks such as discoloration potential, presence of toxic elements in the material composition, difficult handling characteristics, long setting time, high material cost, absence of a known solvent for this material, and the difficulty of its removal after curing. Biodentine™ (Septodont, Saint Maur des Fossés, France) is amongst these materials and is claimed to be used as a dentine restorative material in addition to endodontic indications similar to those of MTA. Biodentine™ powder is mainly composed of tricalcium silicate, calcium carbonate and zirconium oxide as the radiopacifier, whilst Biodentine™ liquid contains calcium chloride as the setting accelerator and water reducing agent[16]. Like MTA, Biodentine™ shows apatite formation after immersion in phosphate solution which is indicative of its bioactivity.

Studies have shown that current methods of cleaning and shaping root canals produce a smear layer that covers the instrumented walls. This layer contains inorganic and organic substances that include fragments of odontoblastic processes, microorganisms, and necrotic materials. The smear layer consists of a superficial layer on the surface of the canal wall approximately 1 to 2 mμ thickness – smear on - and a deeper layer packed into the dentinal

tubules to a depth of up to 40 mμ – smear in. The components of the smear layer can be forced into the dentinal tubules to varying distances. This can occur as a result of the linear movement and rotation of instruments and because of capillary action generated between the dentinal tubules and the smear material[17].

Current methods of root canal instrumentation produce a layer of organic and inorganic material (smear layer) that may also contain bacteria and their byproducts. This layer covers the instrumented walls and may prevent the penetration of intracanal medications into the dentinal tubules and may affect close adaptation between root canal filling materials and the root canal walls[17].

Considering the aforementioned observations, it seems reasonable to suggest that removal of the smear layer can result in a more thorough disinfection of the root canal system and the dentinal tubules, which would ensure a better adaptation between the obturation materials and the root canal walls. Current methods of smear layer removal include chemical, ultrasonic, and laser techniques[17].

Sodium hypochlorite (NaOCl) is the most widely used irrigation solution in endodontics. Currently available evidence strongly indicates that NaOCl is the irrigant of choice[18]. In endodontic therapy, NaOCl solution are used in concentration varying from 0,5% to 5,25%[19]. It dissolves pulpal remnants (vital and necrotic pulp tissue), organic compounds of dentine, the organic components of the smear layer[20] and is characterised by having strong antibacterial activity with comparably short contact times[18,19]. The tissue-dissolving capability of NaOCl is significantly better than all other commonly used irrigants[21]. Moreover, neutralisation or inactivation of lipopolysaccharides has been reported with NaOCl. However, NaOCl is not able to remove the smear layer[22].

So, the use of an organic tissue solvent (NaOCl) intercalated - final rinse - with a chelating agent (17% EDTA) allows for removal of both surface debris and the smear layer, which is not successful when one of these irrigation solutions is used alone[23].

Chlorhexidine is a synthetic cationic bis-guanide that consists of two symmetric 4-cholorophenyl rings and two biguanide groups, connected by a central hexamethylene chain[24]. CHX is a positively charged hydrophobic and lipophilic molecule that interacts with phospholipids and lipopolysaccharides on the cell membrane of bacteria and then enters the cell through some type of active or passive transport mechanism[25]. Its efficacy is because of the interaction of the positive charge of the molecule and the negatively charged phosphate groups on microbial cell walls[26,27], thereby altering the cells' osmotic equilibrium. This increases the permeability of the cell wall, which allows the CHX molecule to penetrate into

the bacteria. CHX is a base and is stable as a salt. The most common oral preparation, CHX gluconate, is watersoluble and at physiologic pH, it readily dissociates and releases the positively charged CHX component[24]. At low concentration (0.2%), low molecular weight substances, specifically potassium and phosphorous, will leak out of the cell. On the other hand, at higher concentration (2%), CHX is bactericidal as precipitation of the cytoplasmic contents occurs, which results in cell death[26].

One treatment protocol that has been suggested to prevent root canal reinfection in vitro is medication with chlorhexidine gluconate (CHX). CHX is a broad spectrum antibacterial agent whose antimicrobial efficacy equals that of the conventional root canal irrigants and medicaments. Unlike the conventional medicaments, the positively charged molecules of CHX can adsorb onto the dentin and prevent microbial colonization on the dentin surface for some time[28].

Dentine may uptake several elements released from bioactive materials, and such a phenomenon may cause chemical and structural dentine modification resulting in acquisition of higher acid resistance and remineralisation[29]. The element incorporation by adjacent dentine may also be regarded as an indicator of the material's bioactivity. However, information is limited regarding how the elements released from different calcium silicate-based materials are incorporated in the dentine in contact with these materials. Thus, the aim of this study was to compare the interface between two calcium silicate-based endodontic materials and root canal dentine conditioned with three different irrigation protocols.

MATERIAL AND METHODS

This study compared two commercially available calcium silicate cements: Biodentine™ (Septodont, Saint-Maur-des Fosses, France, Lot: B06158) and White ProRoot MTA (MTA; Dentsply Tulsa Dental, Tulsa, OK, USA, Lot: 12001879).

SAMPLE PREPARATION

Sixty-six recently extracted human teeth - with straight, single roots fully developed apexes and without caries - were shortened coronally to a standardized length of 14mm and stored in Chloramine-T 0,5% until required. The crowns of each tooth were sectioned at the cement-enamel junction using a water-cooled diamond disc at low speed. The working length for root canal instrumentation was verified by the direct method, and the length was determined to be 1 mm shorter than the actual length of the root.

All teeth were prepared with ProTaper rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland) up to a finish file F4. Apical patency was maintained through the preparation with a size 15 K-type file. One millilitre of 3% CanalPro NaOCl (Coltene/Whaledent, Langenau, Germany, Lot: 4341492) was used for irrigation between each instrument with a notched needle. A different final rinse was made according to the irrigation protocol adopted for each group. Each root canal was dried with paper points.

The roots were divided into three equal experimental groups, of twenty-two specimens each randomly attributed to each group. The materials were mixed according to the manufacturer's instructions and inserted into the prepared root canal specimens by using an MTA carrier and an appropriate root canal condenser.

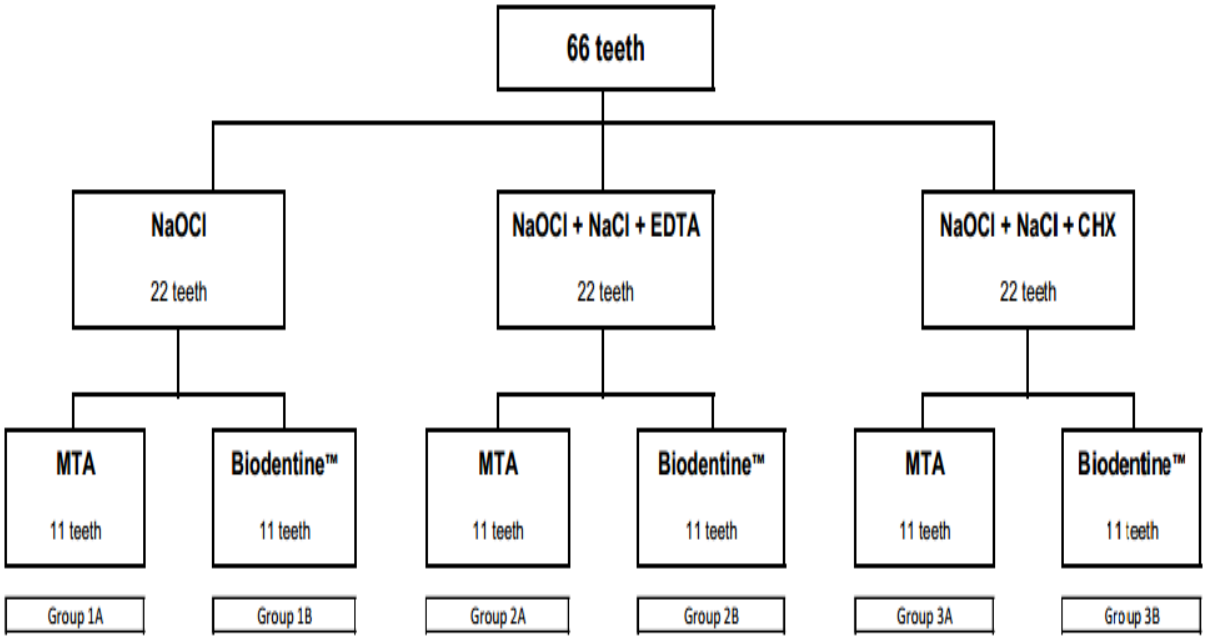

Figure 1 - Study groups and sample number by material and irrigation protocol.

GROUP 1A: In all root canals was made a final irrigation of 1mL of 3% NaOCl and then they were filled with vertical compaction using MTA.

GROUP 1B: In all root canals was made a final irrigation of 1mL of 3% NaOCl and then they were filled with vertical compaction using Biodentine™.

GROUP 2A: In all root canals was made a final irrigation of 1mL of 3% NaOCl followed by 5mL of 17% EDTA for 1 min and 1mL of saline solution and then they were filled with vertical compaction using MTA.

GROUP 2B: In all root canals was made a final irrigation of 1mL of 3% NaOCl followed by 5mL of 17% EDTA for 1 min and 1mL of saline solution and then they were filled with vertical compaction using Biodentine™.

GROUP 3A: In all root canals was made a final irrigation of 1mL of 3% NaOCl followed by 1mL of saline solution and 2mL, 2% CHX for 5 min and then they were filled with vertical compaction using MTA.

GROUP 3B: In all root canals was made a final irrigation of 1mL of 3% NaOCl followed by 1mL of saline solution and 2mL, 2% CHX for 5 min and then they were filled with vertical compaction using Biodentine™

The teeth were stored for 5 days at 37ºC in a moist environment to allow the set of sealers. Each specimen was then sectioned perpendicular to the longitudinal axis of the root using a precision cutting machine (Accutom 50, Struers, Ballerup, Denmark) at 300 rpm. One section of 3mm thick was obtained from the apical third. The specimens were immersed in plastic vials containing Ca and Mg-free PBS (pH 7.2) performing 3x the volume occupied by the specimens, for 5 days.

After this period of time, one specimen of each group was sectioned longitudinally into two symmetrical pieces. One of the two halves - the one with the material - was processed for morphological observation, element mapping and chemical analysis along the dentine-material interface.

MORPHOLOGICAL AND ELEMENT ANALYSIS

The samples were fixed to preserve their native structure with glutaraldehyde – chemical fixation. The samples were then dehydrated by passing the specimens through a graded series of ethanol-water mixtures to 100% EtOH for 10 minutes.

The specimens were mounted on an aluminium stub using carbon sticky pads, sputter-coated with a 300-Å-thick gold layer with an ion coater and analysed using an energy-dispersive X-ray spectroscopy with an image observation function (Phillips XL 20, Philips, Eindhoven, Netherlands with an integrated EDS system from EDAX, EDAX Inc., New Jersey, USA).

For the morphological observation, the outermost dentine layers of the dentine-material interface were analysed under SEM/EDS at an accelerating voltage of 10 kV.

RESULTS

Scanning electron microscopy (SEM) analysis revealed the presence of an 'interfacial layer' and a tag-like structure within the dentinal tubules along the dentine-material interface (Figs. 2, 3 and 4). In this picture we can analyse the tag-like structure resulting from a sample that belongs to group 2B where smear layer has been removed after a final rinse with EDTA. The size of tag like structures is consistent with dentinal tubules size.

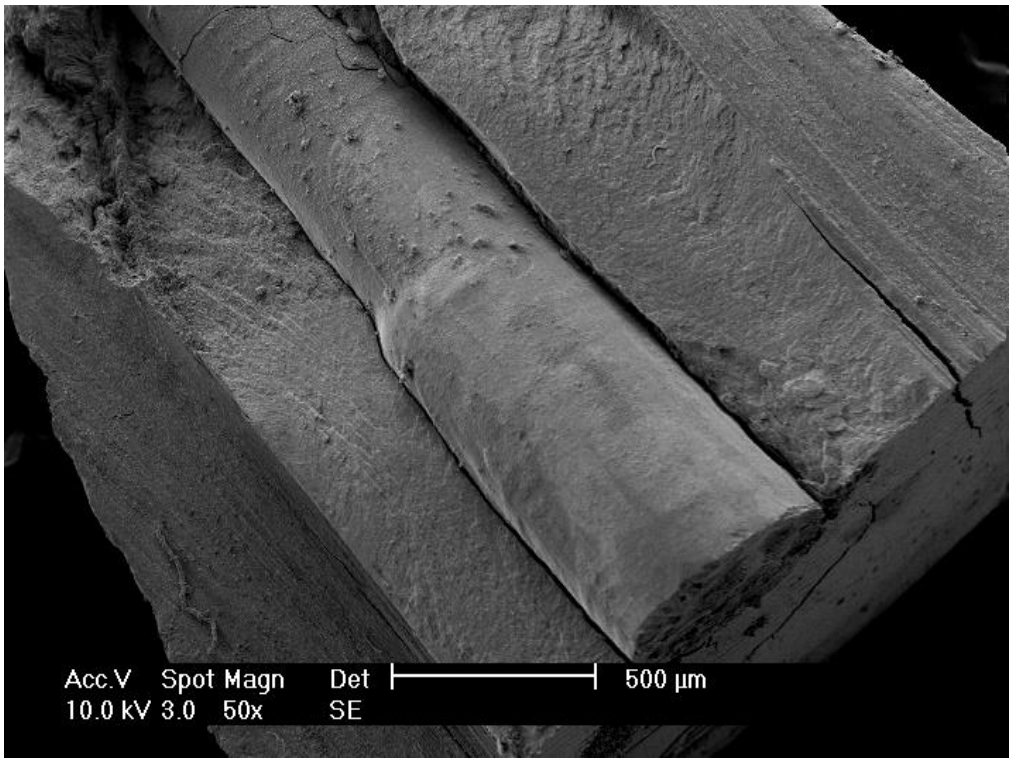

Figure 2 - Low magnification from a cross-sectioned tooth filled with Biodentine™ (Group 2B)

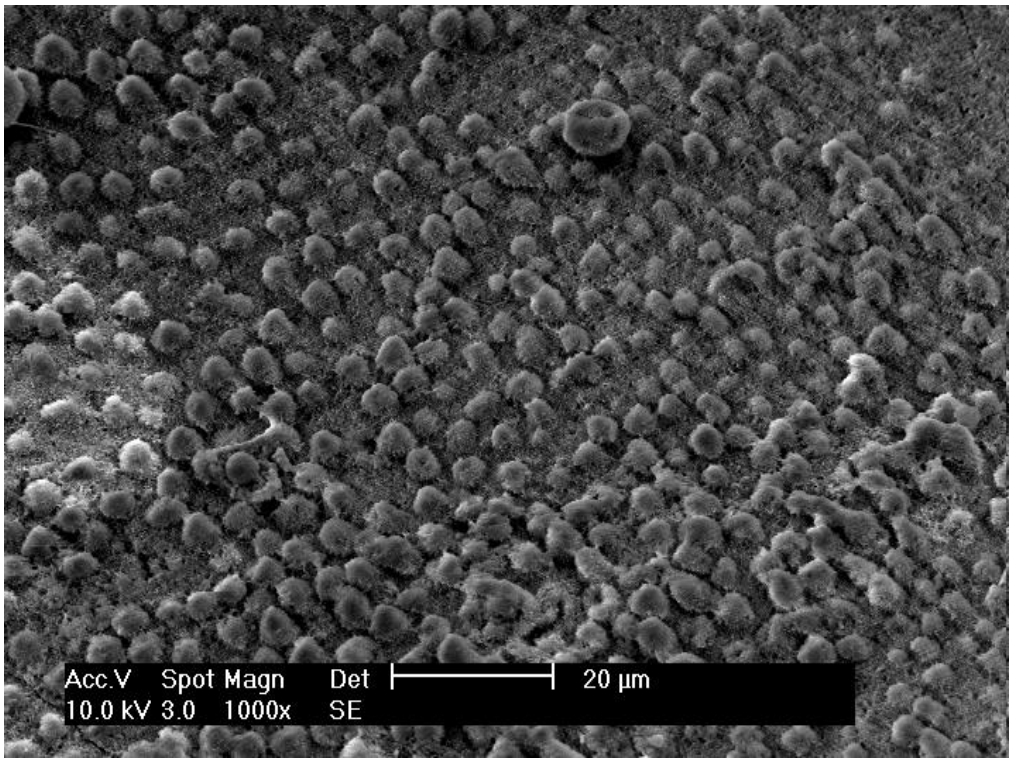

Figure 3 - Representative SEM micrographs of Biodentine™ surface after 5 days of phosphate-buffered saline immersion in group 2B.

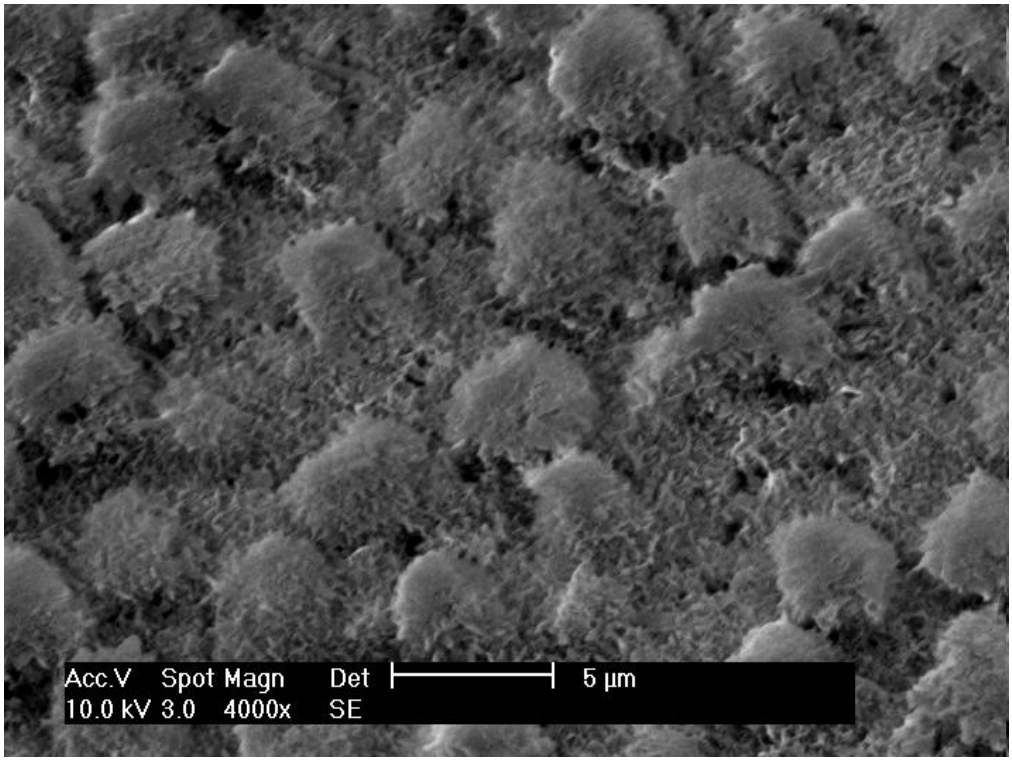

Figure 4 - Previous picture enlarged. It is possible to observe the presence of tag like structures.

We can see in all groups an apatite crystal formation along the interface and within the interfacial dentine in all groups (Figs. 5, 6, 7, 8, 9 and 10). Groups 1A and 1B presented many prismatic/cubic crystals on the surface (Figs. 5 and 6). Groups 2A and 2B showed a mix of prismatic/cubic crystals areas and amorphous irregular outer surface (Figs. 7 and 8). Groups 3A and 3B showed an amorphous irregular outer surface free from visible deposits (Figs. 9 and 10).

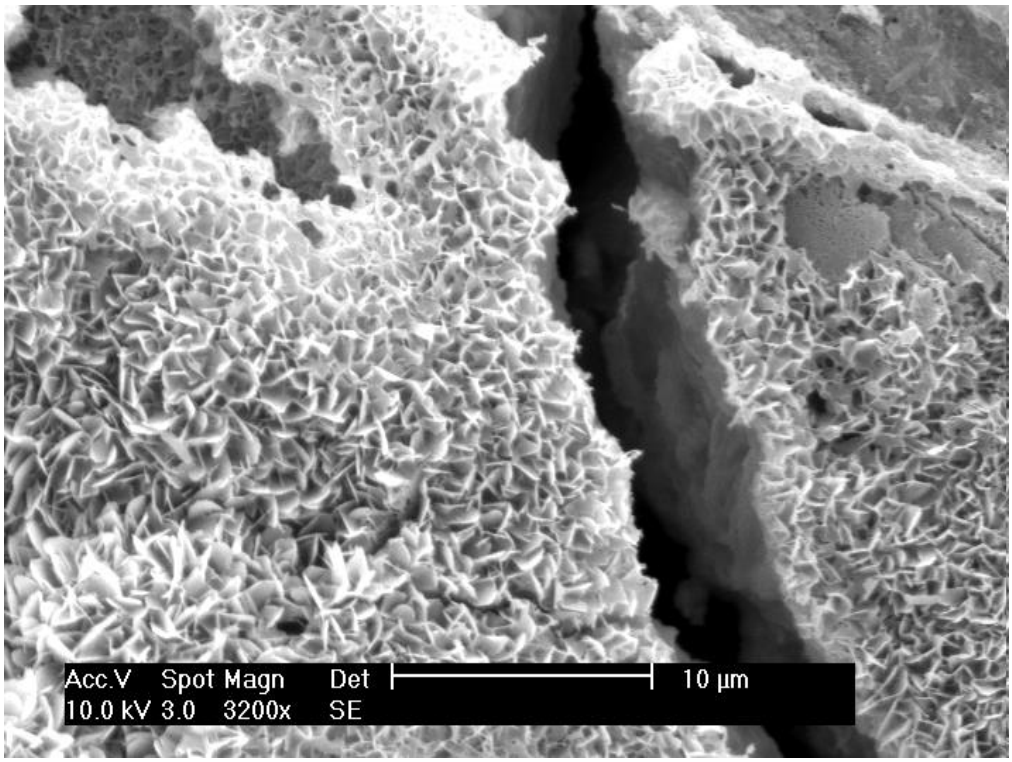

Figure 5 - Apatite crystal formation in group 1A.

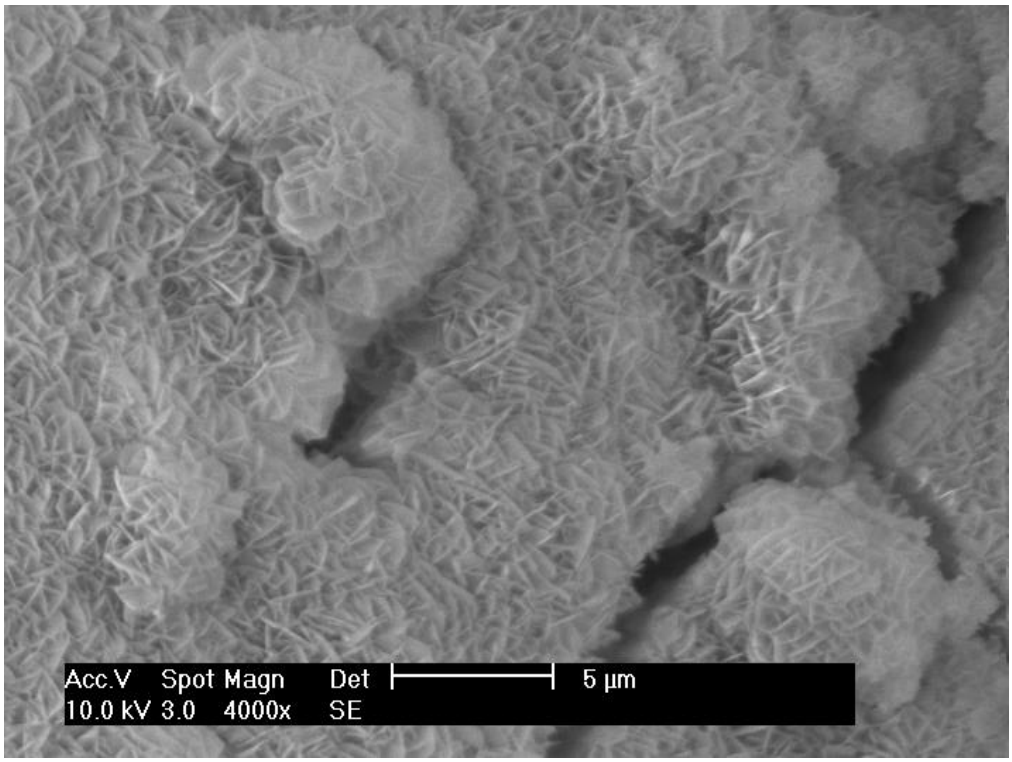

Figure 6 - Apatite crystal formation in group 1B.

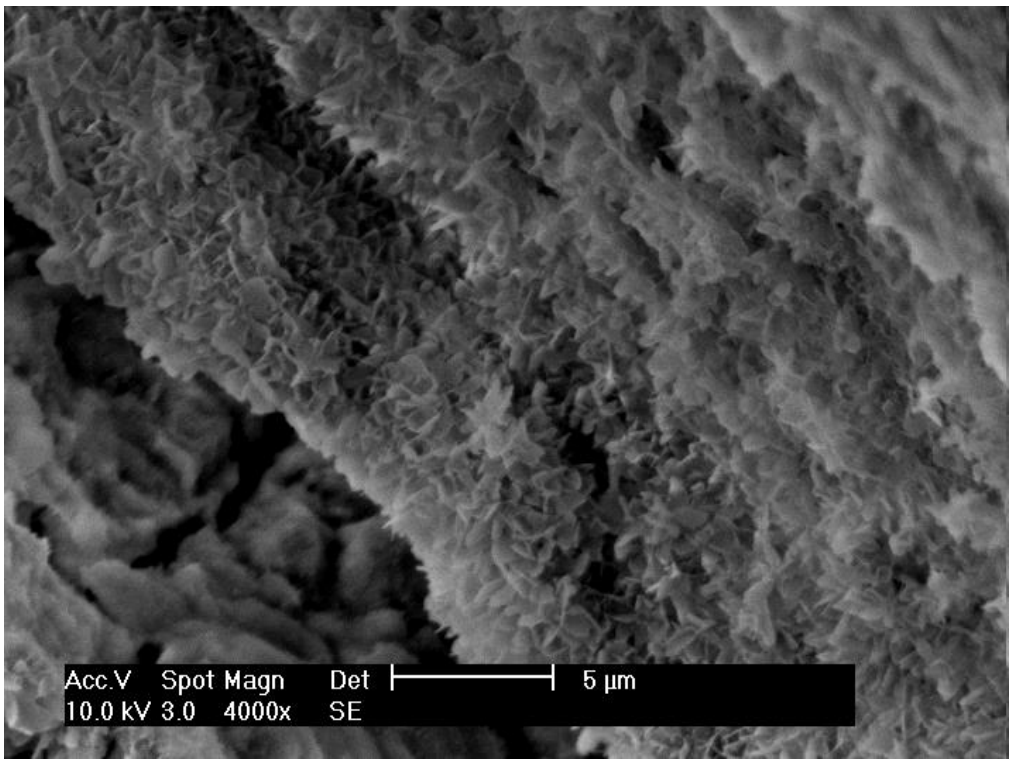

Figure 7 - Apatite crystal formation in group 2A.

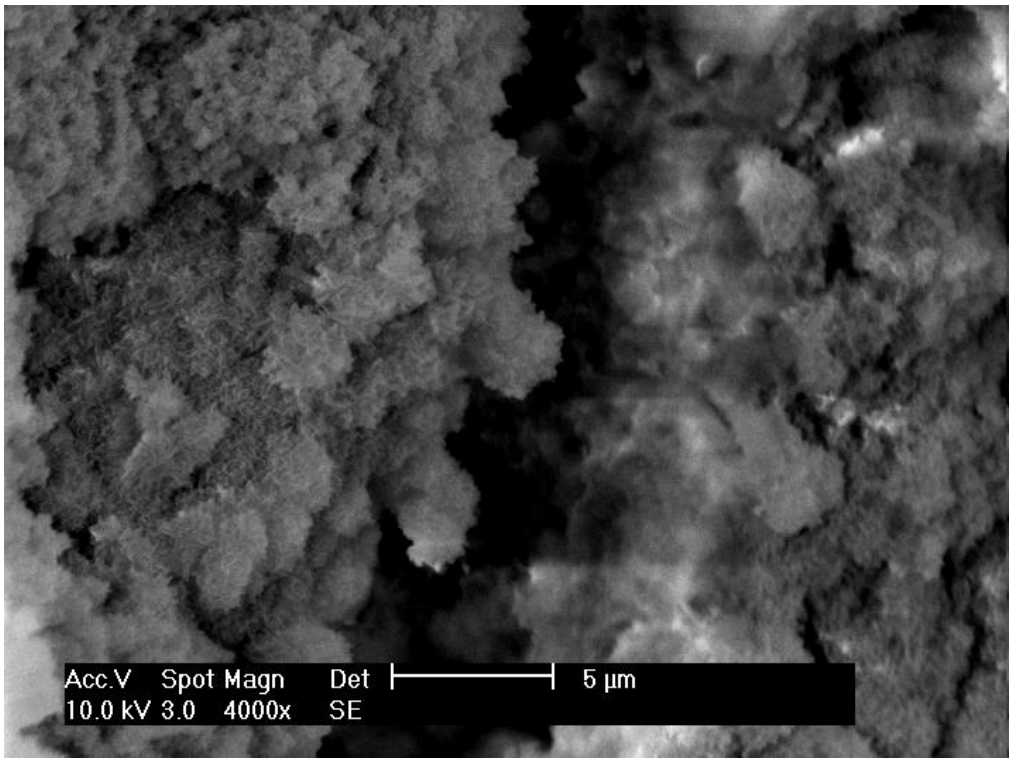

Figure 8 - Apatite crystal formation in group 2B.

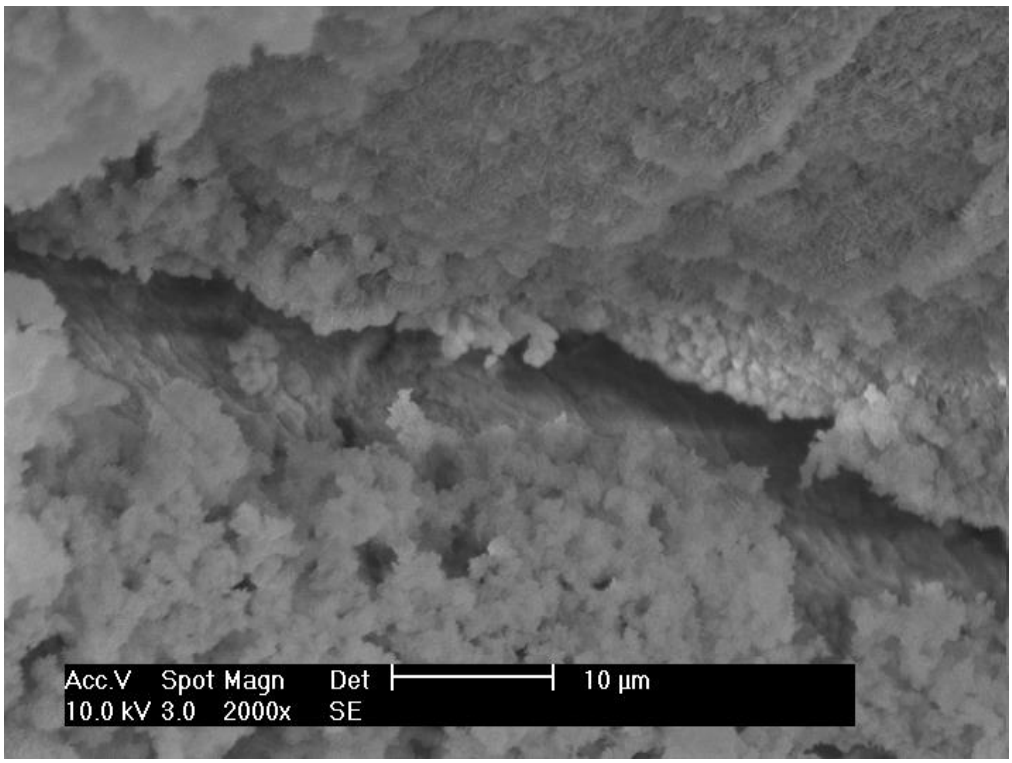

Figure 9 - Apatite crystal formation in group 3A.

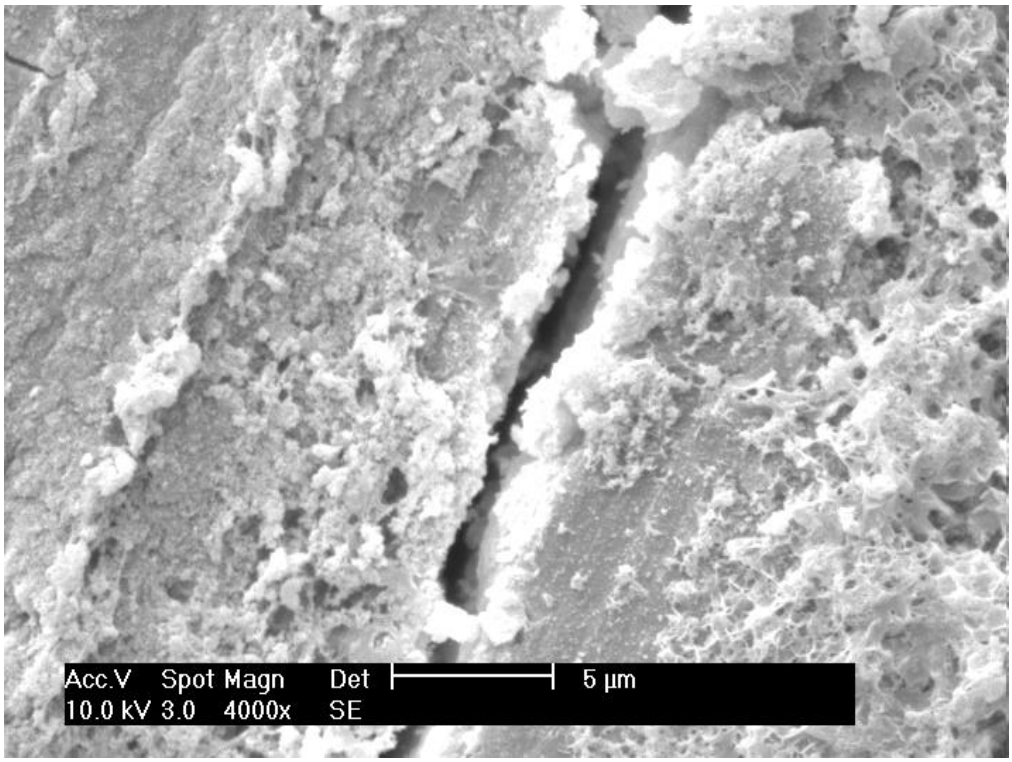

Figure 10 - Apatite crystal formation in group 3B.

Samples were evaluated by EDS for the presence of the compounds Ca, O, P and C at the material dentinal wall interface. Element mapping revealed that Ca- and Si-rich dentine areas were observed along the dentine-material interface. The Ca depth was constantly larger than the Si depth. Moreover, Biodentine™ specimens constantly showed larger Si values versus the corresponding MTA specimen values (Table I).

Table I - Principal composition of the interfacial dentine layer of all six groups (atomic %).

	Phosphate-buffered saline immersion					
	5 days					
	Groups					
Elements	Group 1A	Group 1B	Group 2A	Group 2B	Group 3A	Group 3B
Ca	7.06	6.03	5.30	13.32	6.77	4.45
O	56.82	57.30	52.05	73.59	81.35	50.73
P	9.18	9.85	6.30	11.85	10.70	8.38
C	26.43	26.12	36.15	0.00	0.00	35.09
Si	0.50	0.70	0.19	1.24	1.18	1.34
Ca/P	0.77	0.61	0.84	1.12	0.63	0.53

n = 1

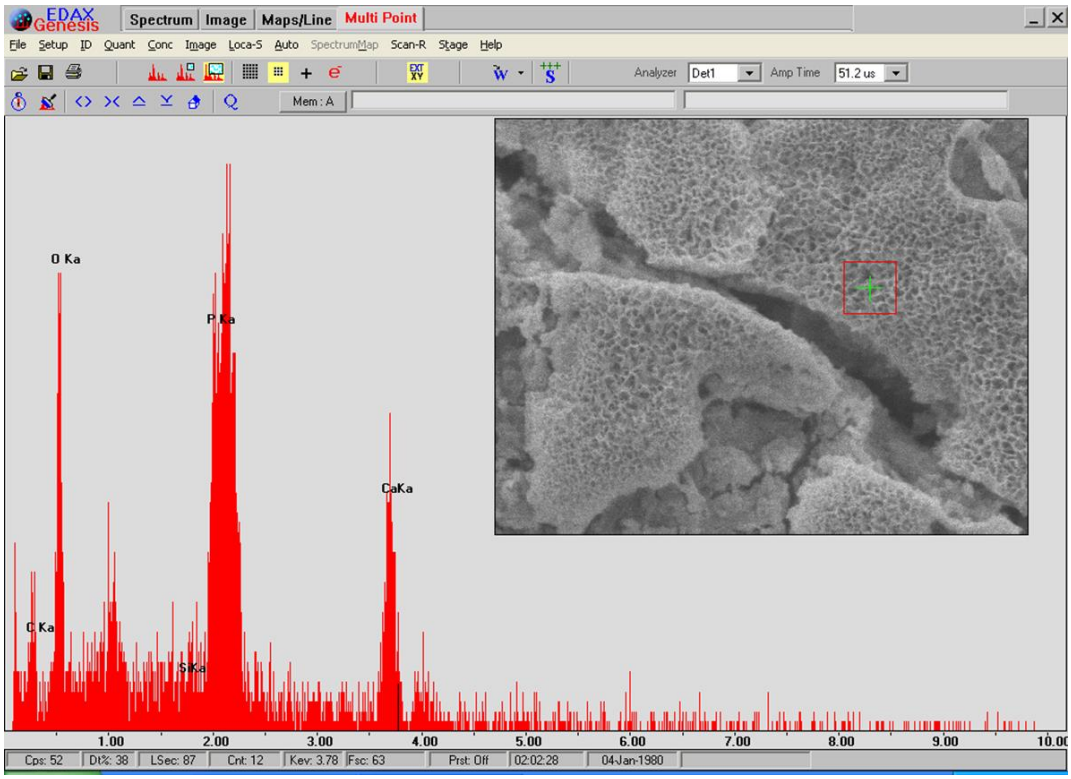

Figure 11 - EDS spectrum of group 1A. The red square in the picture represents the area that was analysed.

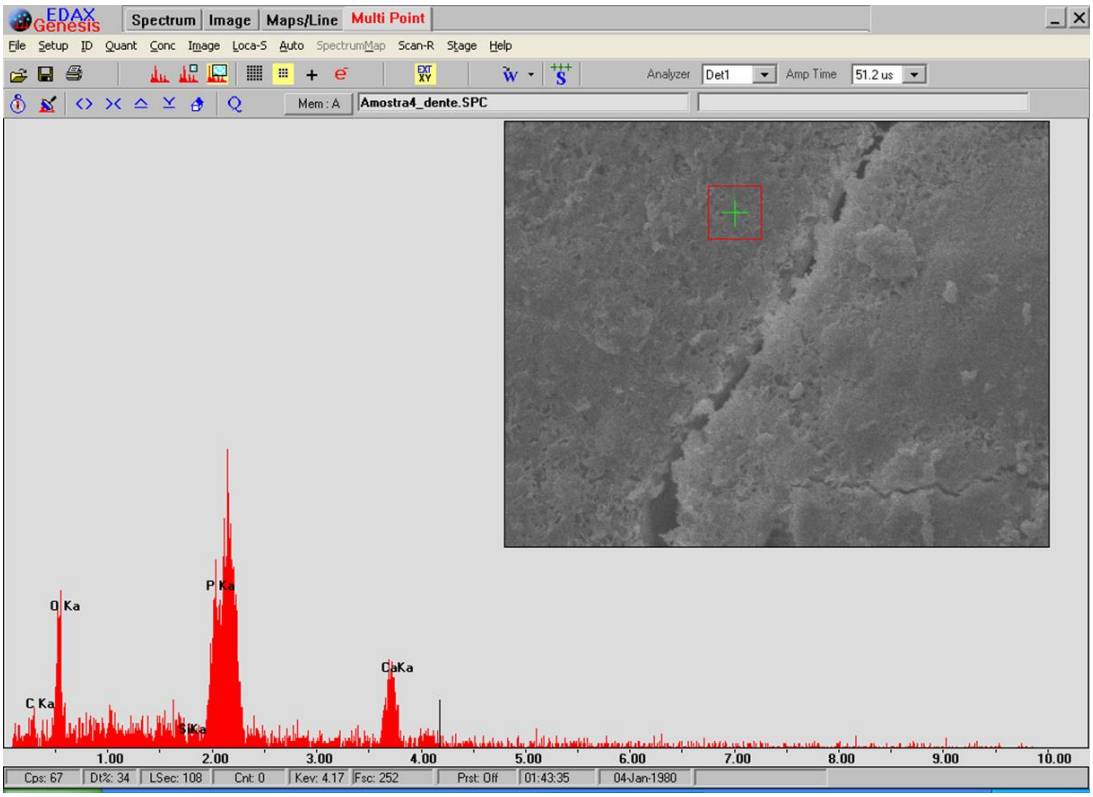

Figure 12 - EDS spectrum of group 1B. The red square in the picture represents the area that was analysed.

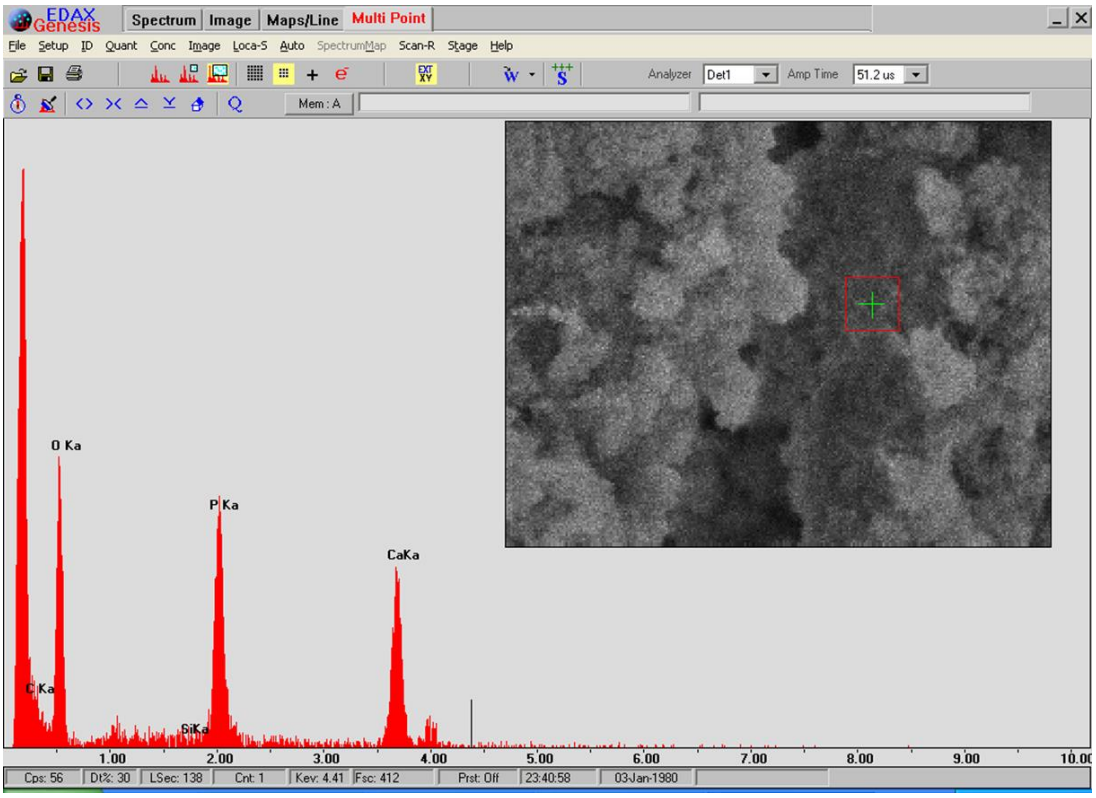

Figure 13 - EDS spectrum of group 2A. The red square in the picture represents the area that was analysed.

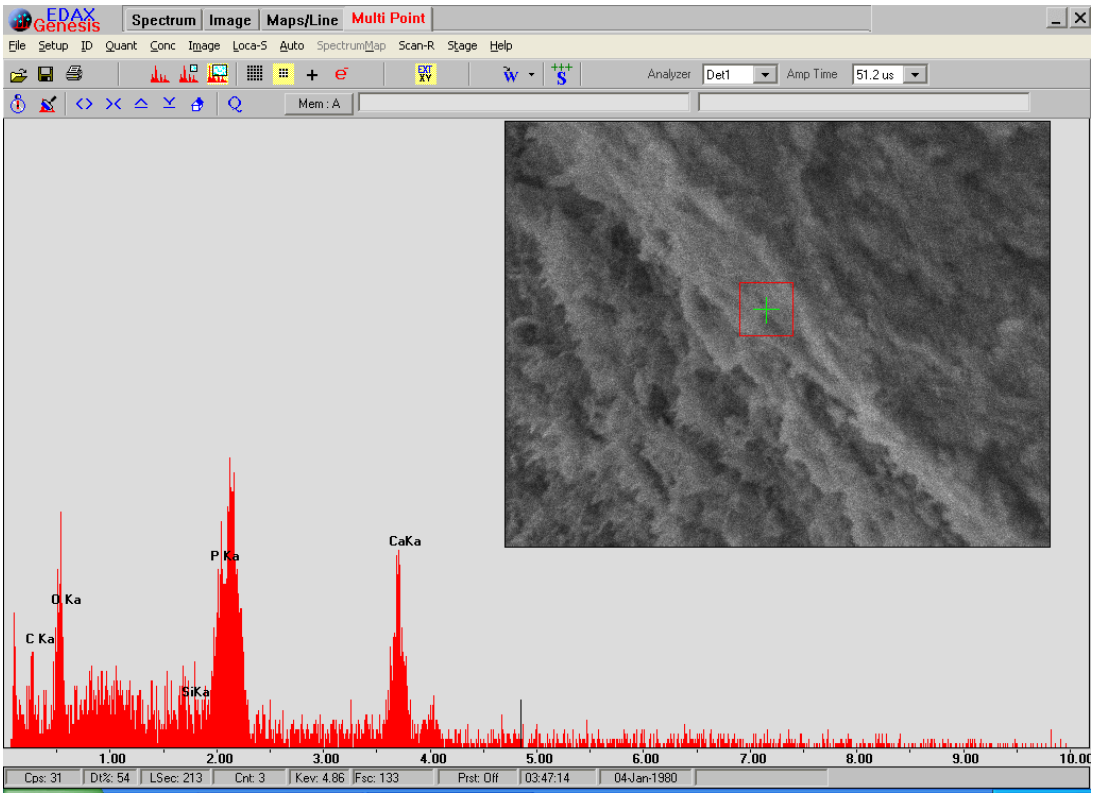

Figure 14 - EDS spectrum of group 2B. The red square in the picture represents the area that was analysed.

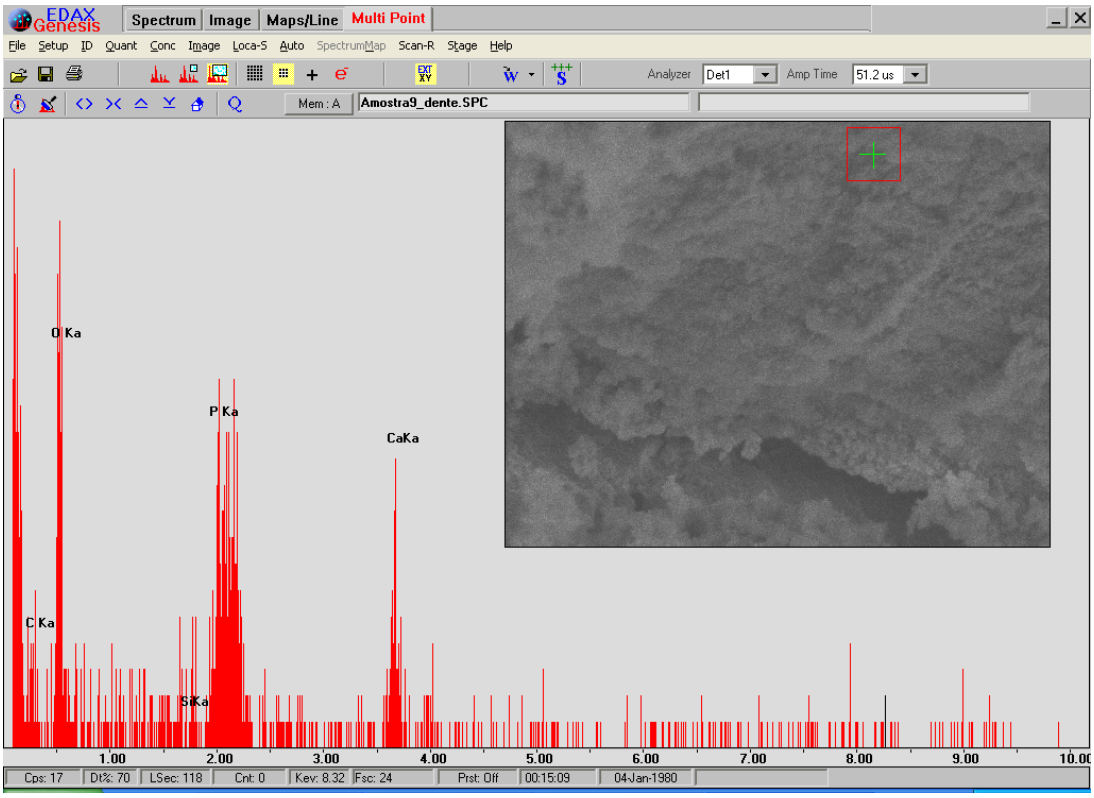

Figure 15 - EDS spectrum of group 3A. The red square in the picture represents the area that was analysed.

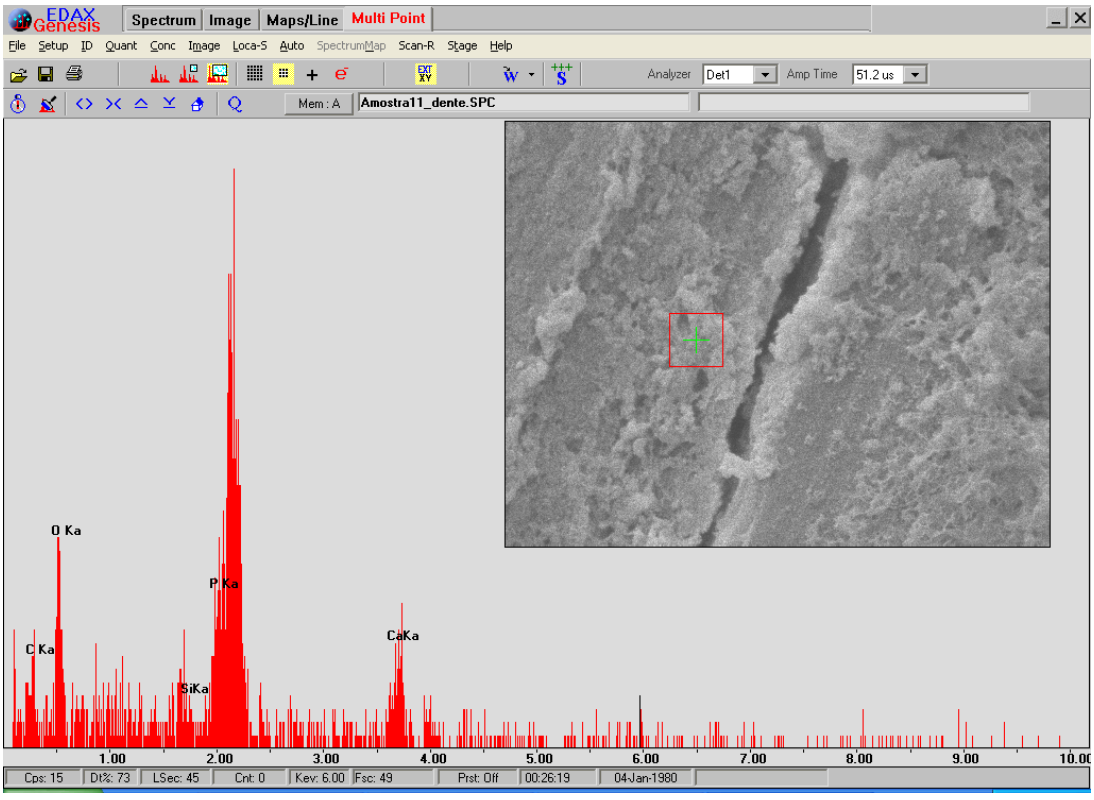

Figure 16 - EDS spectrum of group 3B. The red square in the picture represents the area that was analysed.

Element mapping at 200 µm was made for groups 1A and 1B revealing that The Ca and Si depth was larger in Biodentine™ than in MTA group (Table II).

Table II – Principal composition of dentine at 200 µm distance from interface dentine layer of group 1A and 1B (atomic %).

	Phosphate-buffered saline immersion 5 days	
	Groups	
Elements	Group 1A	Group 1B
Ca	5.64	6.25
O	41.25	51.85
P	7.69	10.41
C	44.82	30.54
Si	0.59	0.95
Ca/P	0.73	0.60

DISCUSSION

Mineral trioxide aggregate is well known for its excellent biocompatibility. Gallego et al. (2008) reported that MTA offered a biologically active substrate for bone and cells[30], and Gandolfi et al. (2008) reported that osteoblasts have a favourable response to MTA[31]. The principle compounds in this material are tricalcium silicate, tricalcium aluminate, tricalcium oxide and silicate oxide[32]. In the hydrated MTA, the main reaction products were calcium silicate hydrate (C-S-H) and calcium hydroxide (CH)[33]. MTA produces a high proportion of calcium ions from CH and C-S-H[34]. The high levels of calcium leached out from the cement account for the biocompatibility of MTA[31,33]. In addition, it is becoming the gold standard to which new root-end filling materials are being compared.

The advantage of using a cement with reduced setting time, like Biodentine™, is the fewer number of visits per treatment when the cement is used for apexification procedures as the tooth can be restored in the same visit. When used as a root-end filling material, the fast set reduces the risk of dislodgement after placement and contamination[35-37].

Ideally, cements should have an antimicrobial action and the ability to stimulate the formation of a mineralized tissue barrier. Antimicrobial activity is related to the release of hydroxyl ions and increasing pH, which generates an unfavourable environment for bacteria to survive. MTA raises the pH of connective tissue and promotes antibacterial effects. On the other hand, the build-up of a barrier is promoted by the release of Ca^{2+} in adjacent tissues that promote hard tissue deposits[38].

The uptake of Ca and Si in the dentine in contact with both Biodentine™ and MTA occurred following PBS immersion. This may represent the biomineralisation ability of these calcium silicate materials promoted by the interaction with dentine in the presence of phosphate-containing solutions[10-12]. Ca and Si uptake most probably causes chemical and structural modification of dentine, which may result in higher acid resistance and physical strength.

The present results also demonstrated that Biodentine™ may have a more prominent biomineralisation ability than MTA, as Biodentine™ specimens showed wider Ca- and Si-rich dentine areas and larger incorporation depths. This could be because of the amount of Ca and Si dissolution that could be larger in Biodentine™ than in MTA.

Chemical analysis of the interfacial dentine layer confirmed increased Ca levels and Ca/P ratios in the Biodentine™ and MTA specimens. This finding is related to Ca incorporation.

It is known that calcium silicate-based ceramic materials commonly exhibit bioactivity to induce bone-like apatite formation[39]; various calcium silicate-based ceramic materials are reported to show such activity, including Portland cement[9,11], dicalcium silicate[40] and tricalcium silicate[41]. Apatite formation on the calcium silicate–based materials may be attributed to the dissolution of portlandite (calcium hydroxide that formed as a result of hydration reactions of calcium silicate materials); this dissolution causes increased pH and Ca2+ ion concentration, which in turn may enhance the super-saturation of phosphate-containing fluid with respect to apatite and, hence, promote precipitation. It has also been described that functional groups, such as Si-OH pre-existing on nanoporous calcium silicate hydrate gel structures formed following hydration reactions, act as nucleation centres for apatite precipitation[42].

The scanning electron microscopy is used in endodontics for ultrastructural analysis of the root dentin subjected to different treatments, the surface of filling materials and the interface filling material/dentine wall[43,44]. This method permits the evaluation of the presence of gaps and tags that are projected into the tubules, allowing a qualitative analysis of root canal obturation[43,45,46].

Studies on the ultrastructure of the MTA–dentine interface after PBS immersion have demonstrated the formation of a mineral-rich interfacial layer and a tag-like structure extending

from the interfacial layer to the dentinal tubules[10,11]. Formation of a 'mineral tag' has also been demonstrated in Biodentine™–dentine interfaces[47]. The present study confirmed these findings and further demonstrated tag-like structures after PBS immersion. Biodentine™ may release a larger amount of Ca and, consequently, produce larger amounts of calcium phosphate precipitates in the PBS environment. That property may have positively influenced the formation of the interfacial layer and the tag-like structure.

Formation of the interfacial layer and tag-like structures may be responsible for good marginal sealing of MTA[3]. This notion is supported by the finding that immersion in PBS decreases marginal leakage of MTA apical plugs[13]. It has also been reported that immersion of MTA in PBS increases push-out strength[12], suggesting that the biomineralisation ability confers the material with greater resistance to dislodgement, most likely through the formation of tags, which constitutes micromechanical anchorage. Biodentine™ is believed to have the potential to exhibit similar characteristics.

A factor that may adversely affect the formation of tags is the presence of smear layer; when present, it prevents the penetration of the material into the tubules[48]. It should be noted, however, that in this study, sodium hypochlorite was used as irrigation solution and a final irrigation with EDTA was only made in groups 1B and 2B. The employment of EDTA reduced the occurrence of this layer and facilitate the formation of tags. Moreover, completely regular walls throughout the root canal are not obtained even after biomechanical preparation. The quality of this preparation depends on the characteristics of the internal anatomy[49].

This study demonstrated the formation of a Si-rich layer in dentine in contact with Biodentine™ and MTA. The amount of Si released from MTA is much smaller than that of Ca[8,10,50,51]. The precise role of Si in hard tissue metabolism remains unclear, although it is believed to play a role in the early bone calcification process[52]. Si is also known to enhance the rate of new bone growth when released from bioactive materials in vivo[53]. Moreover, Si is reported to induce remineralisation of demineralised dentine in vitro[54]. These findings suggest that the release of Si from calcium silicate–based materials may confer additional in vivo bioactivity of these materials.

CONCLUSION

Both Biodentine™ and MTA caused the uptake of Ca and Si in the adjacent root canal dentine in the presence of PBS. The elemental uptake into dentine was more prominent for Biodentine™ than for MTA regardless the irrigation protocol. Apatite crystal formation was

observed in all groups indicating that both materials are bioactive. Tag like structure formation was more evident in groups where EDTA was used.

Further research is required to confirm the consistency of those results and investigate the evolution of the interface dentin/material when samples are kept stored longer time in PBS.

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