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Interaction of hydraulic calcium silicate and glass ionomer cements with dentine

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Key words: mineral infiltration zone, hydraulic cements, glass ionomer, interfacial characteristics, confocal microscopy, atomic force microscopy

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Abstract

RA restoration needs to be placedof missing when there is loss of tooth structure may require the . If the destruction is close to the dental pulp, pulp protection materials need to be placed under the restoration.use of a dentine replacement material to protect the dental pulp. The interaction of a pulp protection material with the underlying dentine is important for the maintenance of a vital pulp. Hydraulic calcium silicate cements and glass ionomers have been reported to exhibit ion exchange at the interface, and this interaction may be enhanced by removal of the smear layer that is formed when dentine is cut. The nature of the interaction and effects of smear layer have not been investigated for hydraulic cements. Biodentine and Chemfil Rock were used as dentine replacement materials in human molar teeth. The dentine was either left untreated or was functionalized by application of 17% ethylene diamine tetracetic acid (EDTA) or 20% polyacrylic acid applied for 1 minute. The interface and dentine interaction were assessed by confocal microscopy (CM) with Rhodamine B, atomic force microscopy (AFM), scanning electron microscopy (SEM) and elemental mapping and by bond strength evaluation. For CM, Rhodamine B fluorescent dye was added to the materials. Two extra groups with Rhodamine B were observed by AFM and SEM to assess the effect of Rhodamine B on the material and interfacial characteristics. The addition of dye to the materials effected the material properties and modified the interfacial zone. The mineral infiltration zone is an artefact caused by specimen preparation for CM thus tThe use of Rhodamine B and CM is thus not recommended for assessment of tooth-material interface. of hydraulic cements and glass ionomers. Dentine functionalization with EDTA improved the material Biodentine contact to the tooth structure so it recommended for both materials. The mineral infiltration zone and material bioactivity were particularly characterized by Ssilicon migration from the material to the tooth structure occurred for Biodentine and the clinical implications of this need to be investigated further.

1. Introduction

Loss of tooth structure (enamel and dentine) is managed by restoring the tooth either by bulk filling with an adhesive composite resin or by replacing the dentine with a dentine replacement material and overlaying this with a restorative material. The role of the dentine replacement material is to protect the dental pulp as loss of pulp vitality will compromise the clinical management.

The interaction of ~~pulp preservation~~dentine replacement materials with dentine is important as t-hese materials need to protect the underlying pulp. It is thus imperative to understand their interaction with the dentine. Both glass ionomer and hydraulic cements have been used as dentine replacement materials. Glass ionomers bond to dentine chemically by ion exchange at the tooth to material interface [1]. There is less literature on the nature of interaction of hydraulic cements with dentine. A recent comparison of both material types demonstrated the interaction of hydraulic calcium silicates ~~demonstrat~~with dentine with what has been termed a~~e ionic exchange with the dentine and a zone of interaction (~~mineral infiltration zone~~) has been identified~~evident at the interface [~~7~~2]. The mineral infiltration zone was observed with Biodentine, a second-generation hydraulic calcium silicate, by confocal laser scanning microscopy. This zone ~~e mineral infiltration zone~~ is reportedly formed by alkaline caustic effect of the calcium silicate cement's hydration products degrading the collagenous component of the interfacial dentin [~~7~~2]. This degradation led to the formation of a porous structure which facilitated the permeation of high concentrations of calcium and carbonate ions, leading to an increased mineralization in this region. This layer was

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shown to be similar to the one formed by glass ionomers where polyacrylic and tartaric acids and their salts characterize the penetration of the glass ionomer cement [~~7~~2]. Conversely, in another study where micro-Raman spectroscopy and field emission gun electron probe microanalysis were used to assess the interface between dentine and Biodentine, interfacial gaps were shown to be filled with calcium phosphate deposits, even though no chemical changes were observed in the adjacent dentin [~~8~~3].

The effect of removal of the smear layer has also not been investigated for hydraulic calcium silicate cements. This layer consists of dentine aggregates of globular subunits approx. 0.05-0.1 micron in diameter. Its composition reflects the composition of the underlying dentine [~~9~~4]. The presence of the smear layer modifies the function of the dentin; It lowers dentin permeability and interferes with attempts to bond dental materials directly to dentine. The smear layer is very acid-labile, and dissolves in oral fluids thus should be removed and replaced with a more stable material overlying the dentine. The effect of smear layer removal on the bonding of glass ionomer cements is well researched [~~10~~5-10~~6~~]. The interaction of hydraulic cements with dentine could also potentially be enhanced by removal of smear layer. ~~The removal of smear layer will widen the dentinal tubules and also modify the dentine chemically. The modified dentine can be bonded to by the hydraulic cements. These materials are very reactive and they will interact with the chemically modified dentin. This interaction has never been investigated.~~

~~as it can trigger responses in the dental pulp leading to a cascade of events where the pulp may either heal or become non-vital. Depending on the response, further pulp management will ensue. Calcium hydroxide has been the material of choice for such procedures. Although it has been used as a pulp dressing material for nearly a century, the mechanism of its interaction with dentine is unclear. It has been suggested that a rise in pH as a result of the free hydroxyl ions may initiate or favour mineralization [1]. Calcium hydroxide is not directly associated with mineralized repair, but is~~

reportedly involved in the initiation process. It has been speculated that the material exerts a mitogenic and osteogenic effect and the high pH combined with the availability of calcium and hydroxyl ions having a synergistic effect on enzymatic pathways and hence mineralization [2]. The high pH may also activate alkaline phosphatase activity which is postulated to play an important role in hard tissue formation [3].

In the past two decades, hydraulic calcium silicates have been introduced for use as pulp protection materials [4]. The mode of action for these materials has been reported to be similar to that of calcium hydroxide [5, 6]. Although calcium hydroxide does not seem to interact with the dentine, hydraulic calcium silicates demonstrate ionic exchange with the dentine and a zone of interaction (mineral infiltration zone) has been identified [7]. The mineral infiltration zone was observed with Biodentine, a second-generation hydraulic calcium silicate, by confocal laser scanning microscopy. The mineral infiltration zone is reportedly formed by alkaline caustic effect of the calcium silicate cement's hydration products degrading the collagenous component of the interfacial dentin [7]. This degradation led to the formation of a porous structure which facilitated the permeation of high concentrations of calcium and carbonate ions, leading to an increased mineralization in this region. This layer was shown to be similar to the one formed by glass ionomers where polyacrylic and tartaric acids and their salts characterize the penetration of the glass ionomer cement [7]. Conversely, in another study where micro-Raman spectroscopy and field-emission gun electron probe microanalysis were used to assess the interface between dentine and Biodentine, interfacial gaps were shown to be filled with calcium phosphate deposits, even though no chemical changes were observed in the adjacent dentin [8].

The cutting of dentine with drills while removing dental caries and preparing it to receive a restoration results in the creation of a smear layer. This layer consists of dentine aggregates of globular subunits approx. 0.05–0.1 micron in diameter. Its composition reflects the composition of the underlying dentin [9]. The presence of the smear layer modifies the function of the dentin; It

~~lowers dentin permeability and interferes with attempts to bond dental materials directly to dentin. The smear layer is very acid-labile, and dissolves in oral fluids thus should be removed and replaced with a more stable material overlying the dentine. The effect of smear layer removal on the bonding of glass ionomer cements is well researched [10-16]. The interaction of hydraulic cements with dentine could potentially be enhanced by removal of smear layer. The removal of smear layer will widen the dentinal tubules and also modify the dentine chemically. The modified dentine can be bonded to by the hydraulic cements. These materials are very reactive and they will interact with the chemically modified dentin. This interaction has never been investigated.~~

~~The effect that a pulp preservation material has on the adjacent dentine is important since changes in the chemistry of the dentine over the dental pulp are responsible for the reparative processes within the pulp.~~ The aim of this study was to characterize the interface between dentine on the cavity floor and hydraulic calcium silicate~~s~~ and a glass ionomer cements to assess the material interaction with the dentine. This was done using three imaging methods namely confocal (CM), atomic force (AFM) and scanning electron microscopy (SEM) ~~along~~ with elemental mapping. Different ways of functionalizing the dentine adjacent to the materials was also investigated.

~~Knowing how these materials interact with the substrate will affect the clinical management of deep carious lesions and gross destruction of tooth structure and will help with the management of the vital pulp~~

The null hypotheses proposed were the following:

- No mineral infiltration zone exists at the tooth to material interface
- No elemental migration occurs at the tooth to material interface
- The Rhodamine B does not affect interaction of the material to the tooth structure
- Dentine conditioning does not improve the material adaptation to the tooth

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2. Materials and Methods

Two materials that have been reported to interact with dentine are investigated. These include aA hydraulic cement developed specifically ~~to be used for pulp protection~~as a dentine replacement material and a glass ionomer cement ~~that is well documented for interacting with the dentine were investigated~~.

- Biodentine (Septodont, Saint-Maur-des-Fosses, France)
- Chemfil Rock (Dentsply DeTrey, Konstanz, Germany)

The tooth to material interface was investigated using three methods namely by investigating imaging the interface by scanning electron (SEM) and confocal microscopy (CM), assessing the elemental migration by energy dispersive spectroscopy (EDS) and investigating the surface characteristics of the interfacial zone by atomic force microscopy (AFM). The bond strengths of the materials to the underlying dentine were assessed by shear bond strength analysis. In addition, the effect of the addition of the fluorescent dye to the materials was evaluated by repeating all the tests with fluorescent dye-doped cements. The effect of dentine conditioning was also investigated. ~~Both materials were mixed according to manufacturer's instructions. They were used to restore medium sized occlusal cavities in molars. Three methods of dentine preparation were empl~~This was done by testing the materials on unconditioned dentine and also on dentine where the smear layer was removed by either dentine primer or a calcium chelator (17% ethylene diamine tetracetic acid – EDTA)~~oyed~~

~~- .No dentine preparation~~

~~- Use of dentine primer – DP polyacrylic acid 20% (GC Europe, Leuven, Belgium)~~

~~- Ethylene diamine tetracetic acid - EDTA, 17% (Endo Solution, Cerkamed, Stalowa Wola, Poland)~~

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~~Both DP and EDTA were applied to freshly cut dentine for 1 minute after which they were rinsed and the dentine surface dried again with a three-in-one syringe.~~

2.1 Tooth preparation for assessment of interfacial characteristics

A human tooth model was used for this research. Caries free human molars were selected from the tooth bank. Ethical approval was obtained from the University of Birmingham ethical committee (IRAS Ref:161303). Medium-sized occlusal cavities measuring 3.5 x 2.5 mm and 3 mm deep were prepared in human molar teeth with a 541-diamond fissure bur. The cavity floors were flattened with an inverted cone bur. The cavities were rinsed with water from the 3-in-1 syringe for 10 s. Three methods of dentine preparation were employed

- No dentine preparation
- Dentine conditioning with dentine primer – DP polyacrylic acid 20% (GC Europe, Leuven, Belgium)
- Dentine conditioning with ethylene diamine tetracetic acid - EDTA, 17% (Endo Solution, Cerkamed, Stalowa Wola, Poland)

Both DP and EDTA were applied for 60s to freshly cut dentine after which they were rinsed with water for 10s and the dentine surface dried again for 10s with a three-in-one syringe.

~~and the dentine was either left untreated or functionalized using either DP or EDTA applied for 1 minute. Surface conditioners were rinsed off with water and the cavities were dried using the 3 in 1 syringe.~~ Both Biodentine and Chemfil Rock were mixed according to the manufacturer's instructions and were used to restore the cavities. The materials were allowed to set for 20 minutes at 37°C and 100% humidity after which the teeth ~~,~~ were stored in Hank's balanced salt solution at 37°C for 30 days prior to testing. A total of 54 teeth were prepared with 27 teeth per material tested, three teeth for each assessment method and three teeth for each dentine conditioning

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method.~~Two sets of specimens were prepared for (i) AFM and (ii) SEM and elemental mapping. The cavities were restored with either Biodentine of Chemfil Rock. A further group was prepared and 0.1% of Rhodamine B was added to both materials prior to placement [17] and these teeth were evaluated by CM. Since it is hypothesised that Rhodamine B effects the material properties and interfacial characteristics, two further groups were added where Rhodamine B was added to the materials and the interfacial characteristics assessed by AFM, SEM and elemental mapping. All the prepared teeth were stored in Hank's balanced salt solution at 37°C for 30 days prior to testing.~~

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2.2 Assessment of interfacial characteristics

The teeth were retrieved from solution and their external surface was wiped to remove excess fluid. They were embedded in a cold cure epoxy resin (Epoxy-fix, Struers, Ballerup, Denmark) and then sectioned along their longitudinal axis mesial to distally using a hard tissue microtome under water coolant. The surfaces were ground and polished with an automatic polishing machine (Buhler, Lake Buff, USA) using 500 and 1200 grit diamond discs under water coolant (Struers, Ballerup, Denmark) and polishing cloths (Struers) with ascending grades of diamond-impregnated polishing ~~pastes~~ liquids (Struers). The sectioned halves were thoroughly washed and also sonicated to remove the debris and polishing slurry.

2.2.1 Confocal microscopy

For CM, ~~six groups were tested. One group restored with Biodentine and another one restored with Chemfil Rock. Both materials had Rhodamine added.~~0.1% of Rhodamine B was added to both materials prior to cavity restoration [11]. Six groups (n = 3) were prepared to evaluate the efficacy of the dentine priming and assess both materials. The teeth were tested immediately after sectioning and polishing to avoid drying out. ~~Three bonding methodologies were used for each material type; unprepared dentine or functionalized dentine with either dentine primer or EDTA.~~

~~After 30 days in HBSS the teeth were sectioned longitudinally and testing was performed immediately following preparation.~~

The specimens were placed in a plastic 35 mm Petri dish and covered with water to get them ready for imaging. The dish was then placed on the stage of an upright Leica DM6 microscope equipped with a Leica TCS SP8 confocal system and imaged with a 40x/0.75 W korr C-apochromat lens directly immerged in the dish. Z-stack images were collected with the LASX software and processed with Fiji (https://imagej.net/Fiji).

2.2.2 Atomic force microscopy (AFM)

~~For this methodology, twelve groups were assessed. The six groups investigated in the confocal microscopy section where Rhodamine B was added to the materials alongside another set of 6 groups with no Rhodamine B added.~~ The morphology of interfacial zone of test materials was observed by AFM (Dimension 3100, Veeco, Cambridge, UK) with a Nanoscope IIIa controller immediately after preparation. A standard AFM probe (NSC15/Al BS, MikroMasch) featuring pyramidal silicon tip (nomial tip radius 8 nm) was used to scan the surface of dental sample in contact mode under water.

2.2.3 Scanning electron microscopy and energy dispersive maps

~~For this methodology, twelve groups were assessed as described in the AFM section.~~ After sectioning longitudinally, the specimens were desiccated overnight by placing in a vacuum desiccator with silica gel, mounted on aluminium stubs and held in place with carbon tape. and were sputter coated with gold. The specimens were then viewed under the SEM (Zeiss). The material to dentine interface was assessed in back scatter mode to obtain elemental contrast. Images at different magnifications were obtained. Energy dispersive maps were obtained to assess the elemental distribution across the interface.

2.3 Evaluation of the effect of Rhodamine on interfacial characteristics

Another t~~T~~wo sets of specimens (n = 9 per group) were prepared for (i) AFM and (ii) SEM and elemental mapping. The same methodology used for the previous analysis was employed. Both materials were assessed and the dentine was also investigated unprepared or functionalised. In this group t~~T~~he cavities were restored with either Biodentine of Chemfil Rock ~~. A further group was prepared~~ and 0.1% of Rhodamine B [11] was added to both materials prior to ~~placement~~cavity restoration. ~~[17] and these teeth were evaluated by CM. Since it is hypothesised that Rhodamine B effects the material properties and interfacial characteristics, two further groups were added where Rhodamine B was added to the materials and the interfacial characteristics assessed by AFM, SEM and elemental mapping. All the prepared teeth were stored in Hank's balanced salt solution at 37°C for 30 days prior to testing.~~

2.~~4~~3 Assessment of bond strength

~~Eighty-four~~One hundred and sixty-eight extracted non-carious human molars were set into epoxy resin (EpoxyCure 2, Buhler, USA) and then polished until a flat coronal dentine surface was achieved using a water-cooled polishing wheel set to 150rpm. The samples were then stored on a damp gauze with the dentine side down until used. The dentine samples were then divided into six groups per material (n = 14) and washed (10s) and dried (10s) using a dental three in one syringe. Samples were either treated with DP or EDTA for 60 s or untreated ~~for 60 s~~ and then rinsed (10s) and dried (10s) again. Immediately after treatment, a 2 mm thick Teflon mould (outer diameter 10 mm, inner diameter 4 mm) ~~place~~ was placed over the prepared surface. Three groups depending on the type of dentine conditioning were prepared and filled with either Biodentine or Chemifil Rock

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prepared according to manufacturers' instructions. Another set was prepared in a similar way but 0.1% Rhodamine B was added to both the Biodentine and Chemfil Rock prior to placement. The samples where then placed into a humidified atmosphere by placing them over a damp gauze with the dentine facing up in a closed container (100% humidity) at 37°C (Hybaid Limited, UK) for 72 hours.

After 72 hours, the specimens were removed and fixed onto a Universal Testing Machine (Instron, 5544, UK) with a bespoke attachment and tested under compression at a strain rate of 1mm/min until the bond between the tooth and the stub failed. The force required to break the bond between the tooth and the stub was recorded and bond strength was calculated by dividing the compression load (N) by the surface area of the stub (cm^2). ~~The procedure was then repeated with the addition of Rhodamine B to Biodentine and Chemfil Rock prior to placement in the Teflon moulds.~~ The penetration of the ~~dye~~ Rhodamine B into the dentine in the groups where the materials were doped ~~into dentine~~ was observed by CM.

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2.4 Statistical analysis

Statistical analysis was performed in Minitab versions 17 using multifactorial analysis of variance (ANOVA). A general linear model ~~(GLM)~~ ANOVA was used to identify significant differences of treatment type and inclusion/exclusion of Rhodamine B ($p = 0.05$). Supplementary one-way ANOVA's were used to separate the difference within these groups ($p = 0.05$).

3. Results

3.1 Assessment of dentine to material interface

The interfacial zone viewed by confocal microscopy for all the groups is shown in Figure 1 and 2. In Figure 1, a z-projection view in the X-Y axis is shown. Figure 2 shows 3D views of the material and tooth interface distributed in the Z-axis where the depth of the interfacial zone can be appreciated. There was tubule penetration for both Biodentine and Chemfil Rock for both untreated and functionalized dentine. The dentine in contact with Biodentine exhibited a zone where the dye was accentuated indicating an exchange between the material and the dentine (arrows in Figure 1A, C, E). This area was more marked when the dentine was functionalized with DP (Figure 1C) and EDTA (Figure 1E). In addition, the Chemfil showed a clear demarcation at the interface for the untreated dentine (Figure 1B). The use of dentine primer caused the development of a structureless zone at the interface (Figure 1D). The use of EDTA resulted in a homogenous continuation of the material into the dentine (Figure 1F).

The interfacial characteristics over 50 by 50 µm, acquired by atomic force microscopy (AFM), are presented in Figure 3. ~~for the untreated and functionalized dentine with no Rhodamine B added, and Figure 4 for all corresponding test groups with Rhodamine B added to the materials.~~ Dashed white lines were introduced as visual guide to indicate the presence of the gap between the material and the dentine. Large triangular features can be found in almost all of the samples. It is clear that the gap becamecomes much more obvious between the dentine and Chemfil Rock (Figure 3B) compared to the Biodentine (Figure 3A). Functionalization of the dentine with DP and EDTA increased the width of the gap especially when EDTA was used with Chemfil Rock. Functionalized dentine enhanced the interaction of Biodentine.~~Addition of Rhodamine B to the materials enhanced the contact (Figure 4 compared to Figure 3).~~

The SEM ~~and elemental maps~~ for all groups are reported in Figure~~s~~ 4~~5~~. ~~and 6 showing the microstructure at the interfacial zones for untreated and functionalized dentine (Figure 5) and the~~

~~same but with Rhodamine B added to both materials (Figure 6).~~ The Chemfil Rock exhibited poor adaptation to the dentine with a large gap at the interface (Figure 4~~5~~B, D, F). The use of dentine primer enhanced the interaction (Figure 4~~5~~D) and the break caused by the vacuum left some material attached to the dentine. Functionalization of the dentine in this case created some gaps (Figure 4C, 4E compared to Figure 4A). ~~The addition of the Rhodamine B to both materials resulted in better material adaptation to the tooth surface and a tighter interfacial zone (Figure 6 compared to Figure 5).~~

Figure~~s~~ ~~7~~ 5 shows the elemental maps of Biodentine and Chemfil Rock with the elements that have penetrated the dentine from the material. Calcium was found both in the materials and in tooth structure. Silicon migrated from the materials into the dentine for both Biodentine (Figure 5~~7~~A) and Chemfil Rock (Figure 5~~7~~B, C). ~~The migration was not that accentuated when Rhodamine B was added to Biodentine (Figure 7C).~~ There were phosphorus deposits at the interface of all the Biodentine specimens (Figure 5~~7~~D) irrespective of the surface conditioning. Chemfil Rock also exhibited migration of aluminium and zinc (Figures 5~~7~~E and 5~~7~~F).

3.2 Evaluation of the effect of Rhodamine on interfacial characteristics

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The addition of the Rhodamine B to both materials resulted in better material adaptation to the tooth surface and a tighter interfacial zone for the Biodentine. This was evident mostly in the SEM analysis (Figure 6~~7~~ compared to Figure 4~~5~~) where even the Biodentine placed on functionalized dentine showed improved adaptation. For the Chemfil Rock the SEM showed very wide-open interfaces and although they appeared apparently tighter when Rhodamine B was added to the material, no conclusions can be made in this regard due to the wide-open interfacial zone. The AFM showed a tighter interfacial zone with better material adaptation to both functionalized and untreated dentine for both material types (Figures 3 and 6)

3.3~~2~~ Assessment of bond strength

All the Biodentine specimens failed during preparation thus the shear bond strength could not be tested. For the Chemifil Rock specimens (Figure 8), the ~~GLM~~ general linear model ANOVA analysis identified significant effects of both the inclusion of Rhodamine B (DF =1, F = 8.83, p = 0.004) and the dentine treatment method (DF = 2, F = 10.27, p = 0.000). The Chemfil Rock exhibited a higher bond strength when it was bonded to dentine pre-treated with dentine primer but only when Rhodamine B was added to the material (P < 0.01). The failure was adhesive for all specimens. Confocal microscopy images (Figure 9) show the penetration of Rhodamine B into the dentine that had already been treated with dentine primer.

4. Discussion

~~Although repair, regeneration, and the nature of the dentine bridge in cases where pulp vitality has been compromised have been extensively researched, there is limited understanding on the interaction of pulp preservation materials and dentine.~~ The interaction of dentine replacement materials to dentine is clinically important as ion leaching from the material will ingress the dentinal tubules and gain access to the dental pulp. Furthermore, gaps between the materials and the tooth structure may become recolonised with bacteria leading to recurrent decay and restoration failure. ~~Although calcium hydroxide is implicated in the cascade of events leading to the initiation of repair mechanisms in the pulp and mineralized tissue formation, the interaction with the dentine is not well documented regardless of its long-standing use in clinical dentistry. Another under-investigated aspect of calcium hydroxide pulp therapy is the benefit of dentine functionalization. No dentine conditioning methods are suggested for priming the dentine prior to the application of calcium hydroxide.~~

In the current study, the nature of the interaction of both glass ionomers and hydraulic cements was investigated. ~~The interaction of glass ionomer with the dentine and its adhesion to tooth structures through chemical bonding is known [14]. This interaction is further enhanced by the use of a dentine primer to remove the smear layer and improve bonding between tooth and glass ionomer [11, 13, 15-16].~~ The interaction of hydraulic calcium silicates with tooth structure is not as well reported as that for glass ionomers. It is believed that hydraulic calcium silicate cements interact with underlying dentine by ~~reported to be similar to that of glass ionomer cement with~~ movement of calcium ions across the interface and the creation of a mineral infiltration zone [~~7~~2] which has been clearly demonstrated using CM. This interaction at the interface has been discredited in a later study [~~8~~3] where no such movement was observed and only deposition of calcium phosphate at the interface was ~~observed~~noted. ~~The current study is in agreement with Li et al. (2016)~~The latter study [3] however, did not investigate the tooth to material interface using confocal microscopy~~[8]~~. Thus, in the current study, it is being postulated that the difference in interfacial characteristics is caused by the dyes used for CM may affect the material interaction with dentine particularly for the hydraulic cements which have been shown to be susceptible to acidic dyes [12].

~~both the effect of dentine functionalization and the interaction with dentine of a hydraulic cement and glass ionomer were investigated. Dentine functionalization needs to be investigated further as it can be beneficial to remove the smear layer prior to application of Biodentine. The use of EDTA is a feasible option for both glass ionomer and hydraulic calcium silicate cements since the application of polyacrylic acid in deep lesions in close proximity to the pulp may be deleterious to the pulp vitality. The use of weak acids with hydraulic cements would also affect their chemistry. Etching of Biodentine for bonding of a composite resin was shown to deteriorate the material surface and also resulted in leakage at the interface between the two materials [18]. Thus, an alkaline primer was chosen for the Biodentine. EDTA is a calcium chelator thus would potentially~~

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~~help remove the smear layer and also functionalize the dentine surface aiding the chemical interaction. The 17% EDTA used in the current study was a solution which is used routinely in endodontics for smear layer removal.~~

~~The interaction of glass ionomer with the dentine and its adhesion to tooth structures through chemical bonding is known [14]. This interaction is further enhanced by the use of a dentine primer to remove the smear layer and improve bonding between tooth and glass ionomer [11, 13, 15-16]. The interaction of hydraulic calcium silicates with tooth structure is reported to be similar to that of glass ionomer cement with movement of calcium ions across the interface and the creation of a mineral infiltration zone [7] which has been clearly demonstrated using CM. This interaction at the interface has been discredited in a later study [8] where no such movement was observed and only deposition of calcium phosphate at the interface was observed. The current study is in agreement with Li et al. (2016) [8].~~

Three assessment methods were used thus used to assess the material to dentine interface. The CM was valuable to track the movement of material across the interface and also map the mineral infiltration zone as in previous research [~~7~~2]. The SEM accompanied by the elemental mapping also allowed the tracking of ~~the ionic exchange~~elemental migration at the interface. ~~In the current study, we added~~ AFM, which to date has not been used to assess these material types was included to investigate the surface characteristics at the interface. All the testing was performed immediately after material preparation without allowing the specimens to dry out for CM and AFM. The CM used in the study also had the advantage of allowing imaging of hydrated samples as opposed to the routine oil immersion objectives used in other studies [2]. It is important to test water-based cements fully hydrated as drying out causes shrinkage and changes the nature of material adaptation to the tooth structure which in fact was clearly shown in the SEM analysis where the materials had to be dried to allow the imaging under vacuum. The confocal microscopy confirmed the previous investigation of the creation of the mineral infiltration zone for the hydraulic

cements [2]. However, the current investigation also confirmed that there was no mineral infiltration zone at the interface as suggested by Li et al. [3] when using elemental mapping and SEM analysis. The AFM further confirmed that the gaps can be caused by drying out. The null hypothesis that no mineral infiltration zone exists at the tooth to material interface could neither be accepted nor rejected although three complimentary methods were used to investigate this. The current study is in agreement with Li et al [3] for the deposition of the phosphorus at the interface of the Biodentine but also elemental migration of silicon, aluminium and zinc was observed. Thus, the null hypothesis of no mineral migration at the interface was rejected.

~~to~~ An attempt at understanding the effect of the Rhodamine B on the material properties and possibly the effect on the dentine and nature of the bonding was thus made by assessing Rhodamine B-doped materials by SEM/AFM and shear bond strength testing. ~~assess~~ These tests clearly showed that ~~the surfaces of the interface and also another group where both materials with the Rhodamine B added were evaluated by SEM and AFM. This was done to investigate the effect that the dye had on the bonding and interfacial characteristics. Another improvement in the current methodology is the use of water immersed specimens for both AFM and CM. This did not allow for dehydration and separation of the material away for the dentine as is evident in the SEM. In addition, the bond strength of the materials to dentine was also assessed to give an indication of the quality of the interaction of the material to dentine.~~ t~~T~~he addition of Rhodamine B to the materials effected their behaviour. A previous study using Rhodamine B with MTA had shown that immersion of MTA in Rhodamine B resulted in a modification of material properties due to the acidity of the Rhodamine and its interaction with MTA [1~~2~~9]. However, the effect of Rhodamine B on the dentine at the tooth to material interface has never been investigated. The SEM analysis clearly showed that the use of Rhodamine B led to a tighter interface particularly for the Biodentine. The shear bond strength testing although not possible for the Biodentine due to debonding during the specimen preparation also confirmed that the Rhodamine B-doping effected the glass ionomer adaptation to the dentine. The dye was shown to be present in the dentine after shear bond strength testing was performed. It

is appreciated that shear bond strength testing has its disadvantages and creates a large standard deviation in the testing as was also observed in the current study. It was however used to show the effect of the dye rather to assess the bond strengths of the materials in the clinical scenario. The null hypothesis that the Rhodamine B does not affect interaction of the material to the tooth structure was rejected.

Removal of smear layer to improve bonding and interaction between tooth and glass ionomer cements has been well investigated in the literature [5-10]. Smear layer may harbour bacteria and it also interferes with the bonding and adaptation of materials in contact with the dentine. ~~both the effect of dentine functionalization and the interaction with dentine of a hydraulic cement and glass ionomer were investigated.~~ Dentine functionalization ~~needs~~ was thus ~~to be~~ investigated further as it can be beneficial to remove the smear layer prior to application of Biodentine. The use of EDTA is a feasible option for dentine conditioning prior to application of ~~both glass ionomer and~~ hydraulic calcium silicate cements ~~since the application of polyacrylic acid in deep lesions in close proximity to the pulp may be deleterious to the pulp vitality~~. ~~The use of weak acids with hydraulic cements would also affect their chemistry. Etching of Biodentine for bonding of a composite resin was shown to deteriorate the material surface and also resulted in leakage at the interface between the two materials [18]. Thus, an alkaline primer was chosen for the Biodentine. EDTA is a calcium chelator thus would potentially help remove the smear layer and also functionalize the dentine surface aiding the chemical interaction.~~ The 17% EDTA used in the current study was a solution which is used routinely in endodontics for smear layer removal. The assessment of efficacy of dentine functionalization using CM may not be accurate as the dye enhanced the effect of the dentine primer when glass ionomer cements were used as dentine replacement material. The AFM also showed tighter material adaptation to the dentine when Rhodamine B was added to the materials. The null hypothesis that dentine functionalization does not improve material adaptation to the tooth was rejected,

The interaction of both glass ionomer and hydraulic calcium silicate cements with dentine occurred through the exchange of various minerals; silicon and phosphorus for Biodentine and aluminium and zinc for the glass ionomer. The silicon migration from hydraulic cements at the interface has already been reported [~~20~~13, ~~21~~14]. The role of silicon on the dentine and the pulpal interaction to the silicon influx are unknown and require further investigation. The Rhodamine B clearly effected the propagation of the material in the dentine creating artefacts. Thus, measurement of depth of material penetration within the dentinal tubules and its clinical relevance to success of a restoration needs to be evaluated with caution as it may not be clinically relevant. The elemental migration is more relevant due to its potential effects on the dentine and pulp.

Conclusions

This study demonstrates that Biodentine interaction with the adjacent tooth structure was through the migration of silicon into dentine and deposition of phosphorus at the interface. Dentine functionalization with EDTA enhances the ~~material~~ Biodentine interaction with the dentine and is recommended for clinical management ~~of pulp capping procedures~~. The use of CM and SEM to investigate the tooth-material interface is not recommended as they may introduce experimental artefacts leading to misinterpretation of data.

Acknowledgements

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Disclosures

The authors declare no conflict of interest

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List of Figures

Figure 1: Confocal micrographs showing a Z-stack projection of interfacial zone of test materials in contact with untreated dentine and dentine pretreated with DP or EDTA. White arrow indicates the presence of mineral infiltration zone.

Figure 2: 3-D rendering of confocal z-stack images showing interfacial zone of test materials in contact with untreated dentine and dentine pretreated with DP or EDTA.

Figure 3: Atomic force microscopy (AFM) height images showing interfacial zone of test materials in contact with untreated dentine and dentine pretreated with dentine primer (DP) or ethylene diamine tetracetic acid (EDTA). Dotted line marks the interfacial zone.

~~Figure 4: Atomic force microscopy (AFM) height images (50×50 µm^2) showing interfacial zone of test materials with Rhoadamine B added in contact with untreated dentine and dentine pretreated with dentine primer (DP) or ethylene diamine tetracetic acid (EDTA). Dotted line marks the interfacial zone.~~

Figure 45: Back scatter scanning electron microscopy images showing interfacial zone of test materials in contact with untreated dentine and dentine pretreated with dentine primer (DP) or ethylene diamine tetracetic acid (EDTA)

~~Figure 6: Back scatter scanning electron microscopy images showing interfacial zone of test materials with Rhoadamine B added in contact with untreated dentine and dentine pretreated with dentine primer (DP) or ethylene diamine tetracetic acid (EDTA).~~

Figure 57: Elemental maps for various elements showing migration from the material to tooth structure (A): Silicon map for Biodentine without Rhodamine B; (B): Silicon map for Chemfil Rock; (C): Silicon map for Biodentine with Rhodamine B; (D): Phosphorus map for Biodentine; (E): Aluminium map for Chemfil Rock; (F): Zinc map for Chemfil Rock.

Figure 64: Atomic force microscopy (AFM) height images (50×50 µm^2) showing interfacial zone of test materials with Rhoadamine B added in contact with untreated dentine and dentine pretreated with dentine primer (DP) or ethylene diamine tetracetic acid (EDTA). Dotted line marks the interfacial zone.

Figure 76: Back scatter scanning electron microscopy images showing interfacial zone of test materials with Rhoadamine B added in contact with untreated dentine and dentine pretreated with dentine primer (DP) or ethylene diamine tetracetic acid (EDTA).

Figure 8: Shear bond strength results showing mean ± SD of glass ionomer cement and glass ionomer cement with Rhodamine B added on treated and untreated dentine.

Figure 9: Z-stack projection of confocal microscopy images of the dentine functionalized with dentine primer that was adjacent to Chemfil Rock prior to shear bond strength testing. The micrographs show clearly the penetration of the dye into the dentinal tubules from the material.

Response to reviewer comments

Comments

Reviewer #1: Thank you for the opportunity to review this manuscript. This study has its merits because the authors are seeking to characterize the interface between dentine on the cavity floor and hydraulic calcium silicates and a glass ionomer using three imaging methods namely confocal (CM), atomic force (AFM) and scanning electron microscopy (SEM) along with elemental mapping. However, to make the work original, rather than a simple comparison between different materials, researchers are encouraged to look at properties that can be really characterized by a sound experimental model.

Responses

The properties that can really be characterised include

- elemental migration across the interface which can be observed by EDS,
- movement of material across the interface as observed by the confocal microscopy which is propagated by the dye as clearly shown in the images after the bonding test. So the dye is effecting the materials and bonding
- the surface characteristics at the interface evaluated by the AFM.

The different techniques were utilised to try to evaluate the interfacial characteristics. The use of the dye and confocal microscopy is not a sound experimental model. Nor is the SEM due to the shrinkage as the materials are water-based. So really and truly it is a bit difficult to evaluate the interface of such materials with dentine.

Comments

Authors are also encouraged to provide substantial information about the rationale for the study design in the Introduction. How to support that: "… The interaction of hydraulic cement with dentine could potentially be enhanced by removal of smear layer…."using an extracted tooth model? And what is the hypothesis that you are trying to validate?

Response

Bonding seems to be a crucial aspect of dentistry. The hydraulic cements have been shown to bond chemically to dentine through the elemental exchange which is a model already shown for glass ionomers. The nature of the interaction is controversial as the confocal microscopy study by Atmeh et al. shows the presence of a mineral infiltration zone but this was discredited in a later study (Li et al.). The current study was set up to test similar materials and use different investigation methods to understand better the nature of the interaction at the tooth to material interface for both materials. Furthermore, since glass ionomer interaction is improved by smear layer removal and this has not been investigated for the hydraulic cements, the effect of dentine conditioning was investigated. The hypothesis have been added to the aims of the study at the end of the introduction and accepted or rejected in the discussion section. Thank you for directing me to this very important point. I was beating around the bush previously.

Comments

Important details are missing in the Materials and Methods and Results sections, such as:
a) How many extracted teeth were used?
b) How they were cut, and for how long they were dried after surface conditioners being rinsed off?
c) What is the n of each group? And how many groups?
d) How could be done an elemental mapping by EDX after the samples were sputter-coated with gold?
e) How to use EDX, AFM, and SEM to analyses the interface if the cavity floor is not 100% flat?
f) For AFM and SEM analyses: How many slices per tooth were done?
g)For the interfacial assessment: How the samples were clean after the polishing pastes?
h) In section 2.4, it is mention p=0.05. Does it mean alfa=0.05?

Response

a) In the assessment of the tooth to material interface by the three methods with 3 methods of dentine functionalization for the two materials 54 teeth were used with n = 3 for each group. For the assessment of the effect of the dye using SEM and AFM, 36 teeth were used. For the bond strength testing 168 teeth were used.
b) They were cut using a hard tissue microtome under water spray. The surface conditioners were applied for 60s. This is not standard methodology for the DP but we wanted to investigate this higher exposure time and also the effect of EDTA on the GIC which is to date never investigated. The teeth were rinsed for 10 s and dried for 10 s.
c) The groups have now been clarified by splitting off the section where the effect of the Rhodamine B was assessed. This is hopefully clearer now. There are six groups per assessment method (3 methods) for the 2 materials and three dentine conditioning methods with n = 3 per group. For the assessment of effect of the Rhodamine doping again 6 groups per assessment method (2 methods).
d) We have used gold sputtering as gold gives better microstructural imaging. The gold peak was eliminated from the elemental analysis and the maps. We did not perform semi quantitative elemental analysis thus the percentage of gold did not interfere with the results obtained
e) We conducted the analysis in different areas to get a representative assessment. For the SEM we chose magnifications that were high enough to eliminate this factor. The cavity floors were flattened with inverted cone but=r to achieve as flat a surface as possible.
f) The teeth were only sectioned longitudinally in half as indicated in the manuscript. So we had 2 halves per tooth. We did not opt for serial sections to avoid material dislodgement as much as possible.
g) Samples were washed under running water and sonicated to clean them off the debris and slurry.
h) Yes the level of significance was 0.05.

All the clarifications have now been added to the manuscript. We thank the reviewer for such a detailed assessment of the methodology. Indeed, a lot of important detail had been omitted in the manuscript preparation.

Comments

When writing the Discussion, please make sure the findings are presented and discussed/supported by information available in the literature. Also, sometimes authors make statements that have already been known for many years Lastly, authors do not show in the discussion section any clinical relevance of this hardworking and expensive study.

Response

The discussion has been rewritten in a more scientific way with the adequate citations, less drama and supported by the literature. Unnecessary obvious statements have been omitted. The clinical relevance has been added to the discussion section. Yes indeed this study has cost considerably and has taken a lot of effort and time to prepare. We would like to pass on the message to the prospective readers which is not only information on how the material interact and how the interaction can be improved but also to be careful with the assessment method chosen due to artefacts.

Figure

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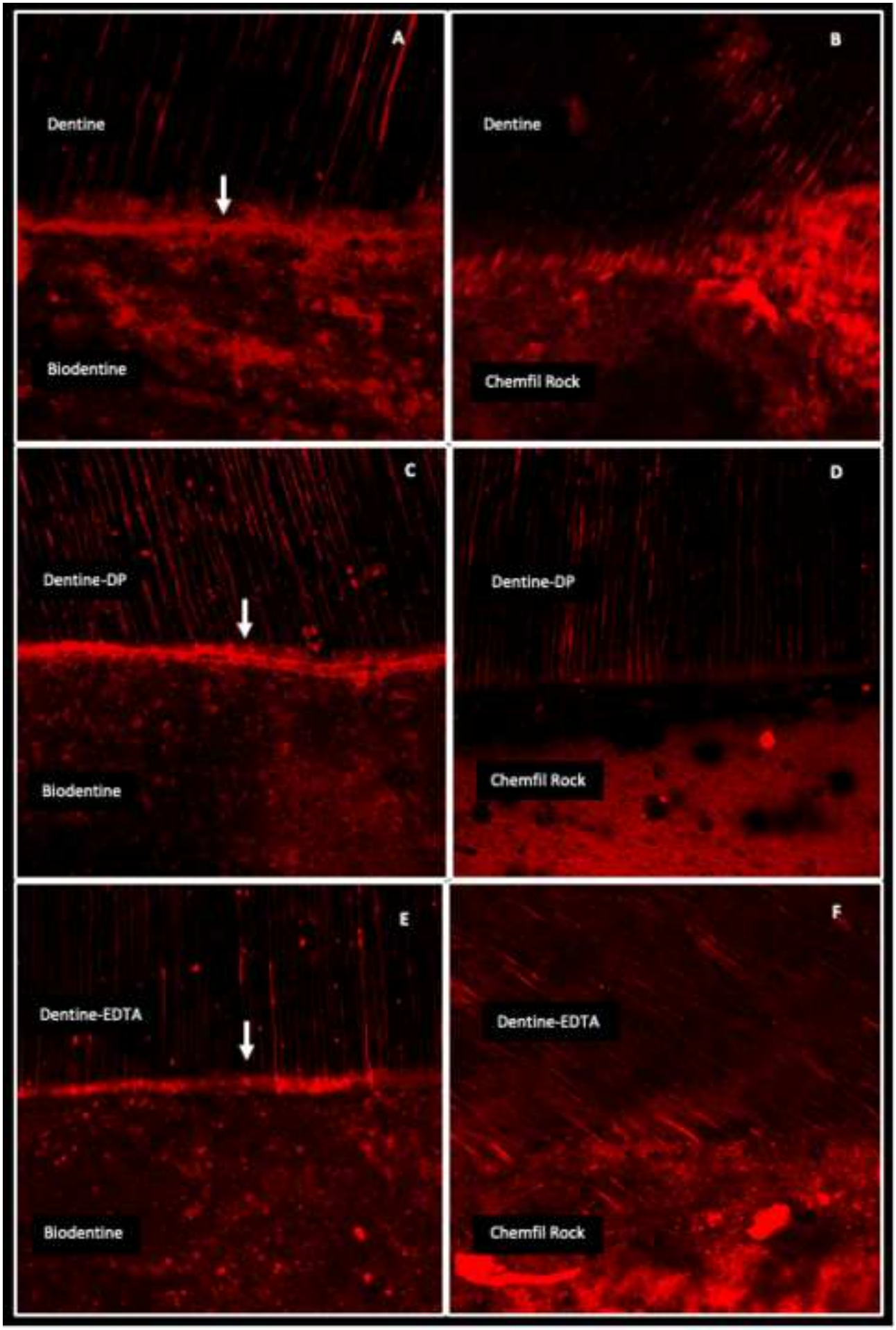

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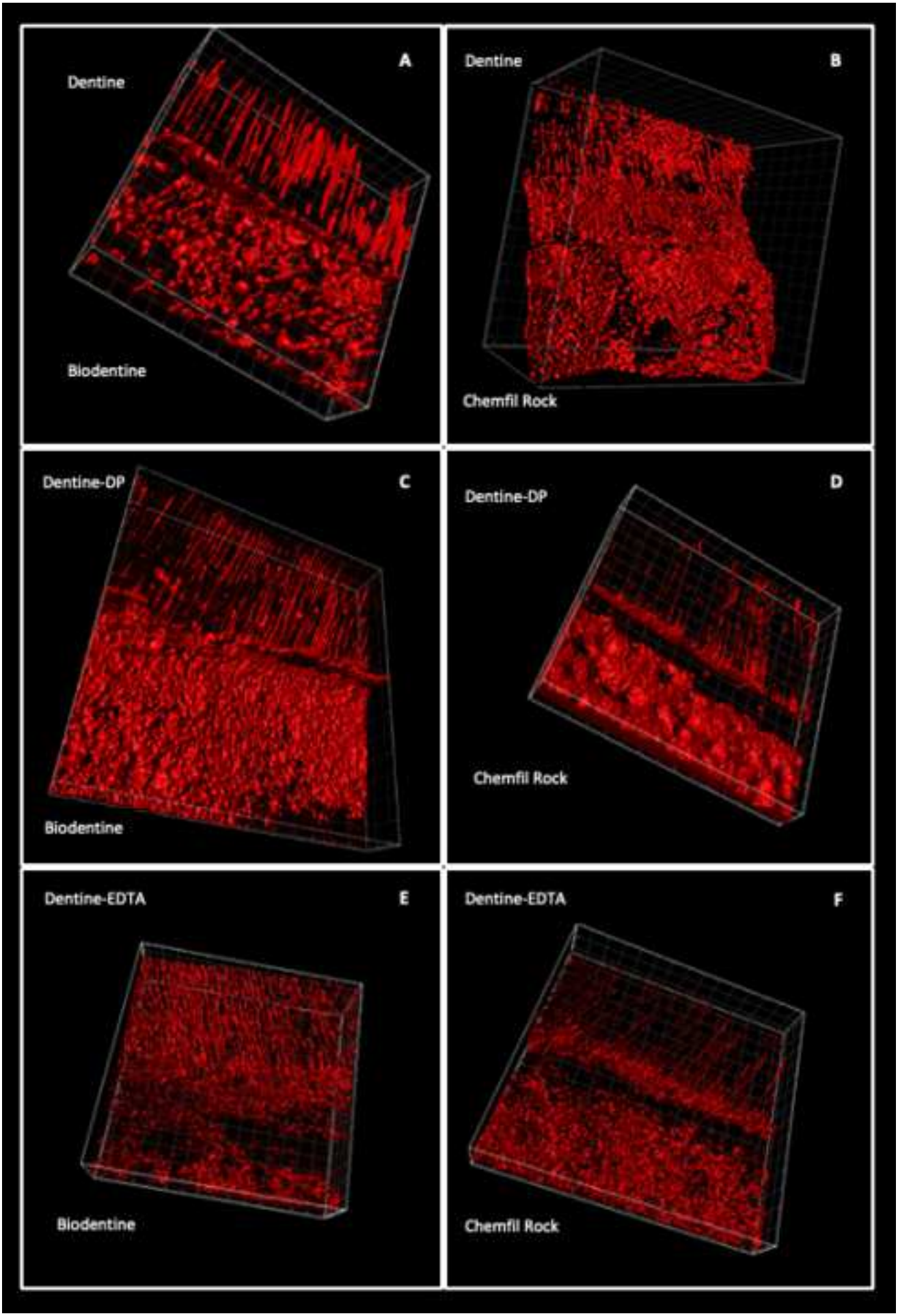

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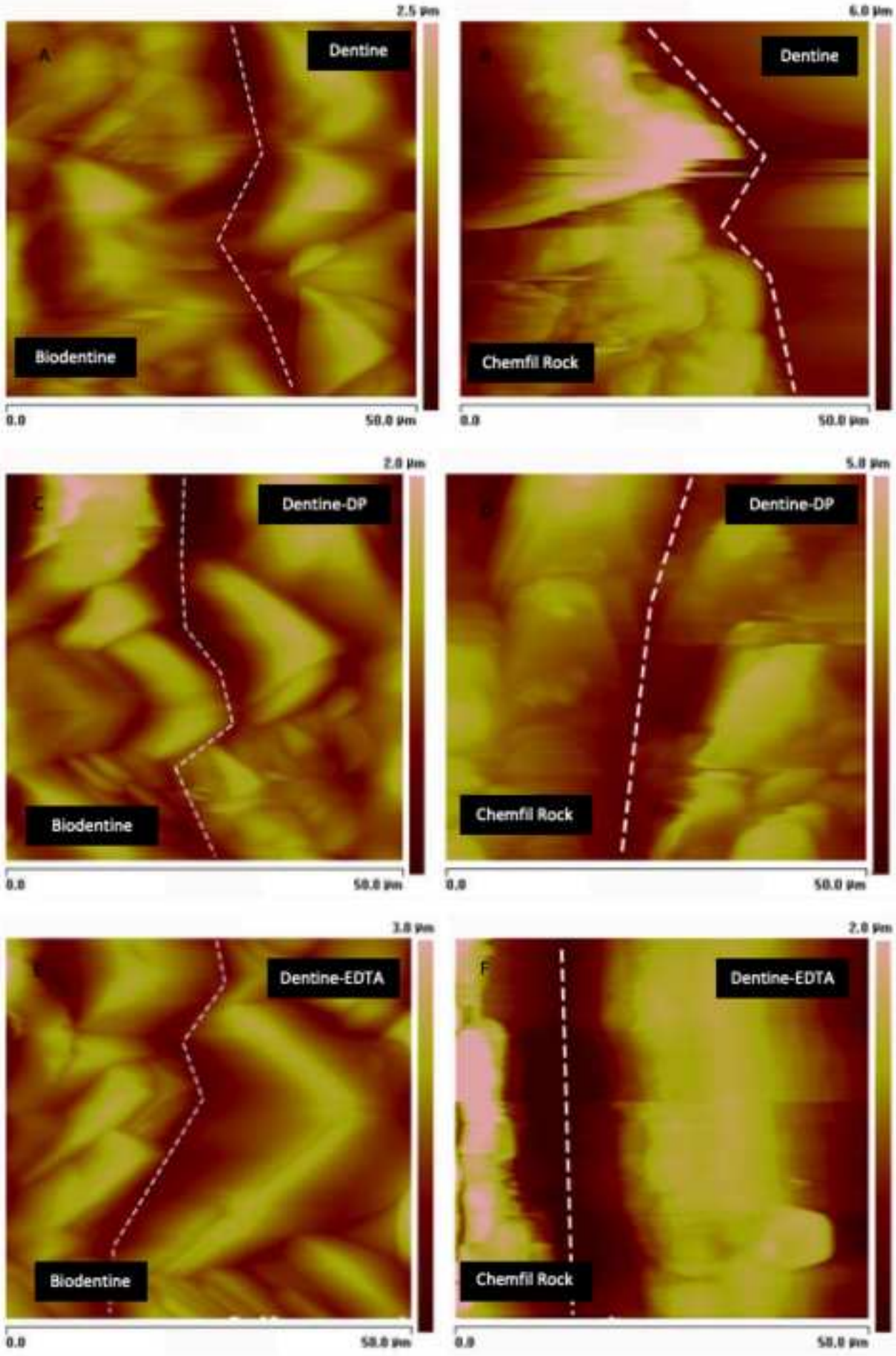

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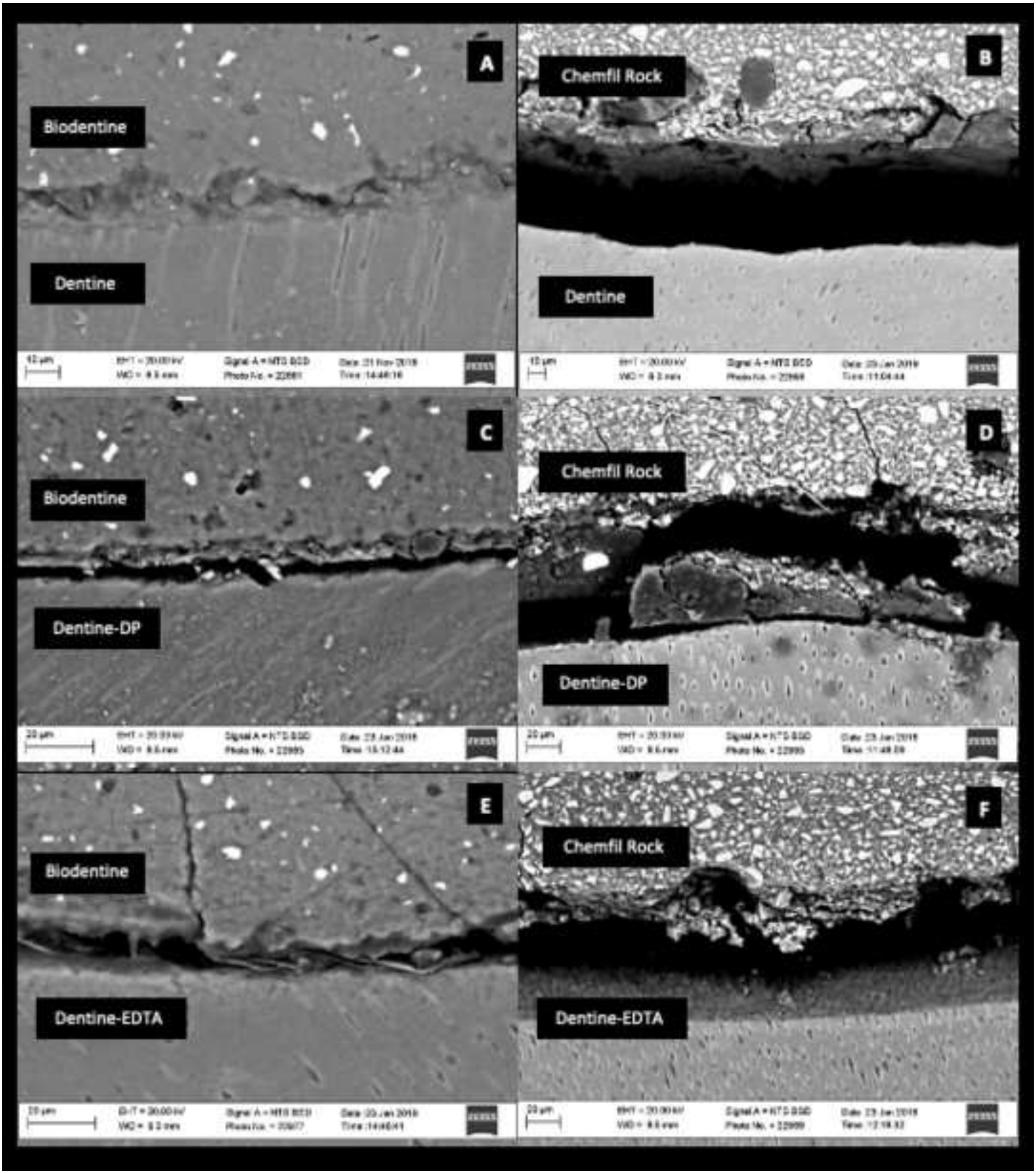

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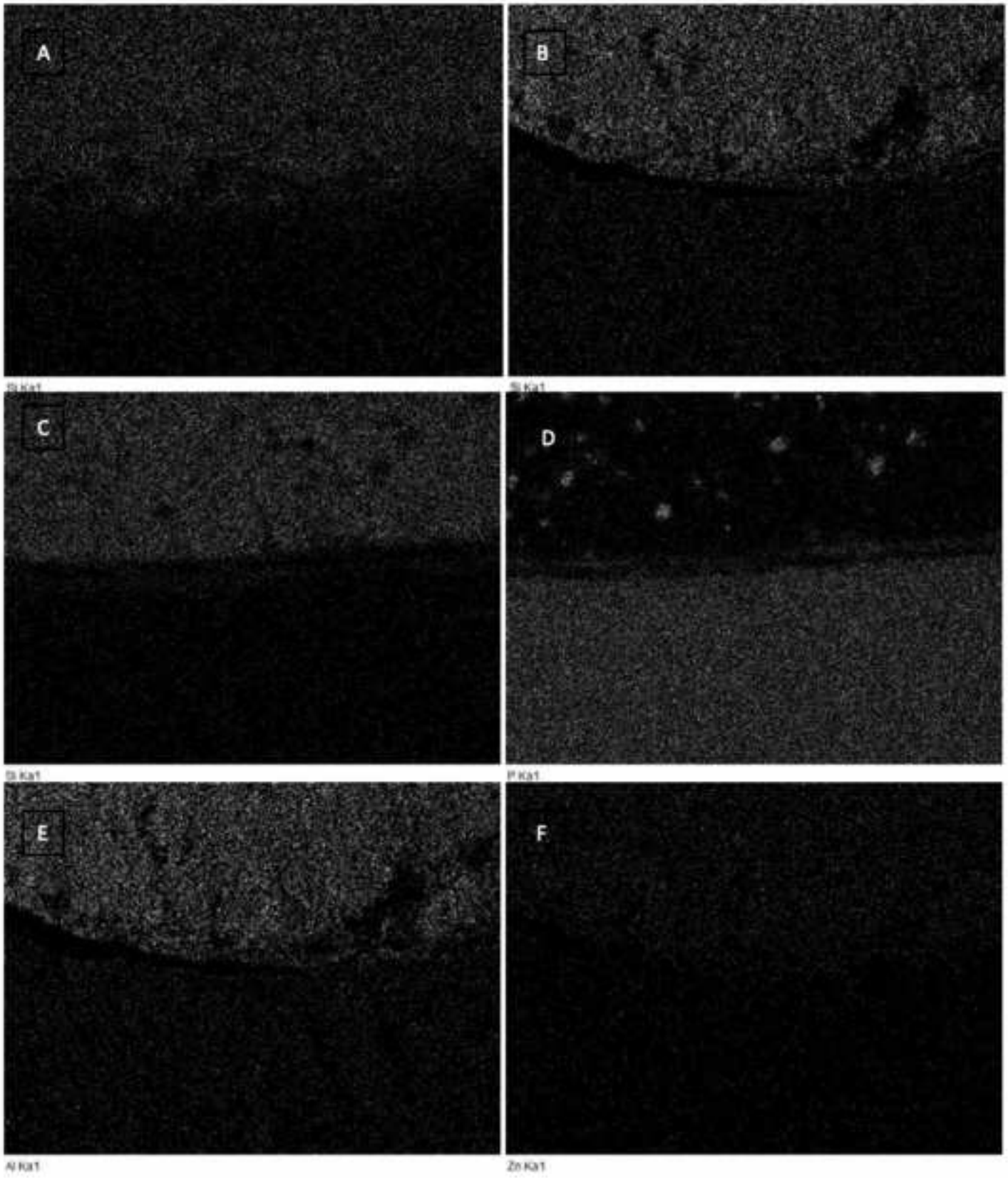

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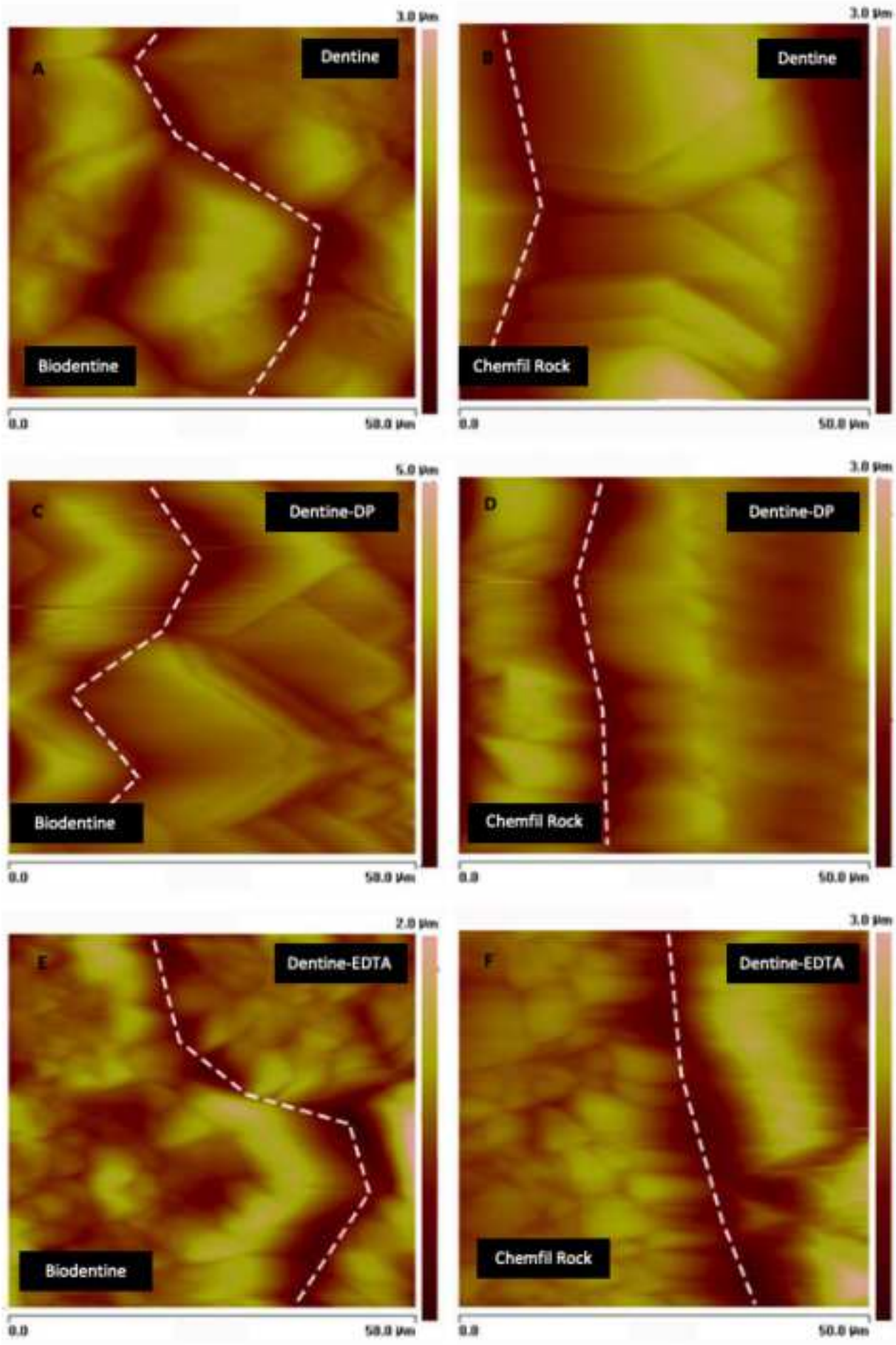

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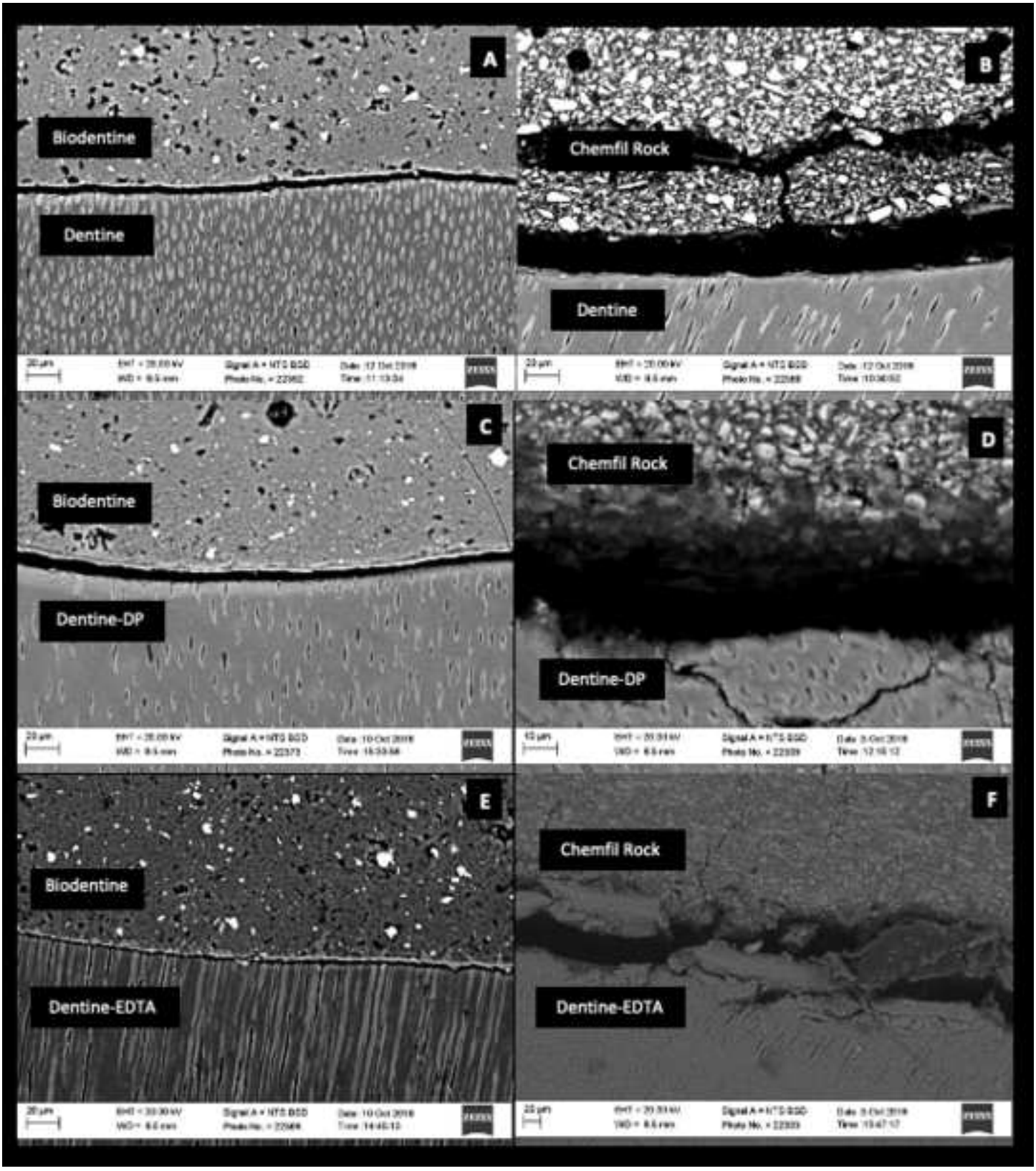

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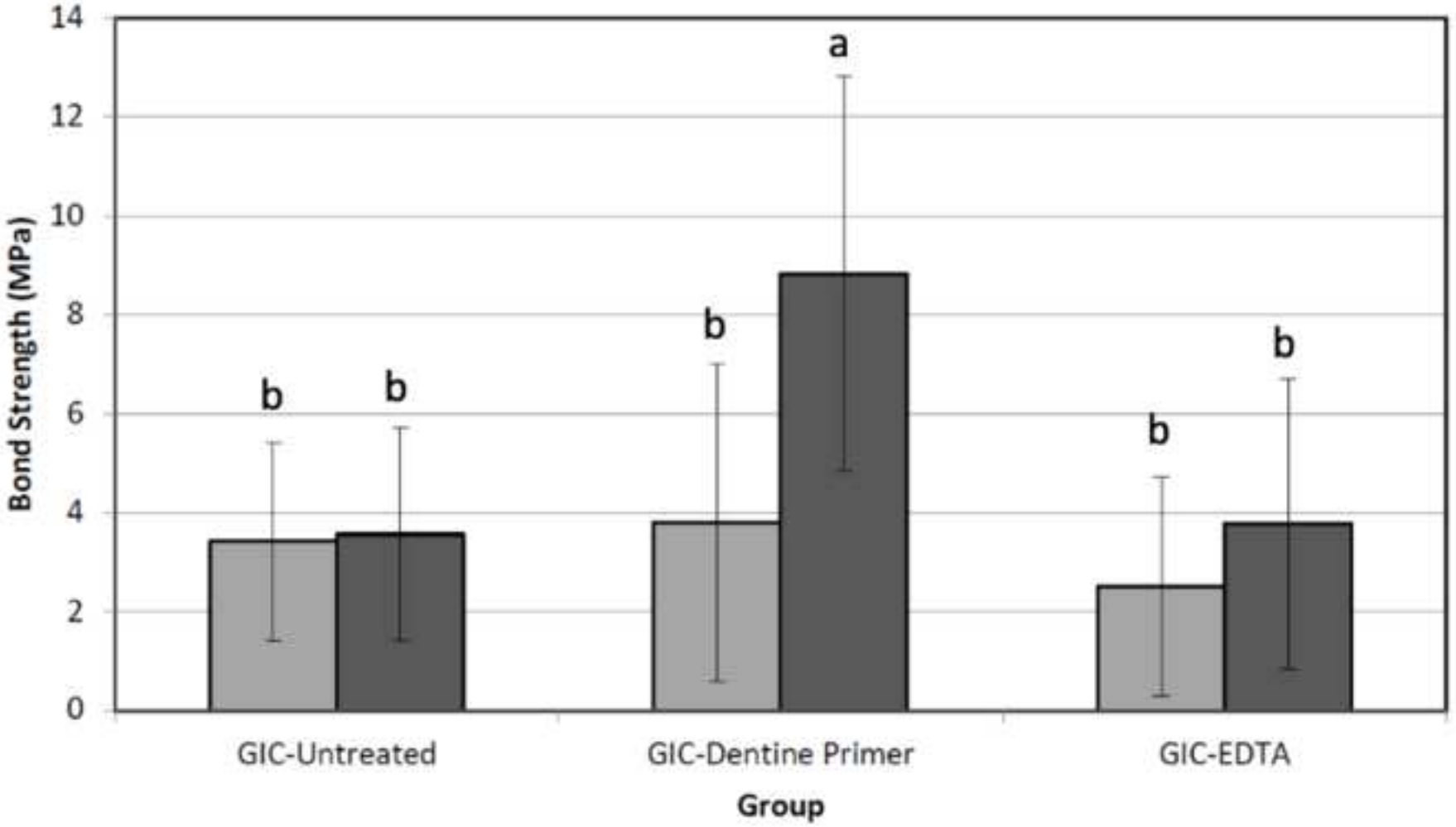

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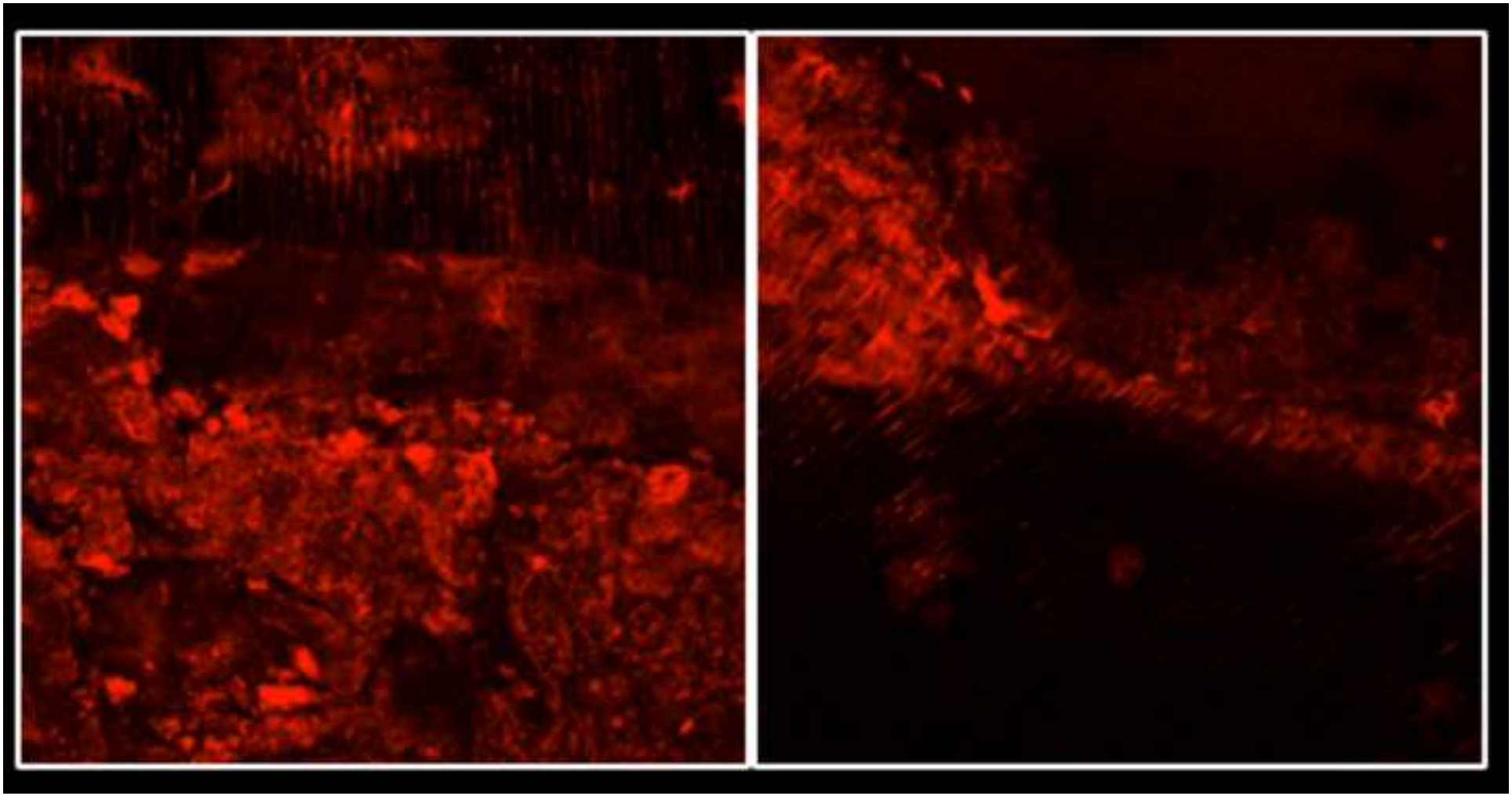

Statement of significance

The interaction of pulp preservation materials with the underlying dentine is important for maintenance of pulp vitality. This interaction is usually based on ion exchange of calcium between the material and dentine and is affected by the smear layer which is formed when the dentine is prepared with rotary instruments. These interactions are studied by various microscopy techniques with the confocal microscopy being very popular as the fluorescent dye clearly maps out the material interaction with the substrate. Unfortunately, the fluorescent dyes affect the interface and the level of penetration. The presence of a mineral infiltration zone, which is linked to the hydraulic tricalcium silicate cements is an artefact. Dentine functionalization with calcium chelators could be an alternative to the use of weak acids and could be more clinically acceptable to avoid pulp damage in deep cavities.

***Declaration of Interest statement**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: