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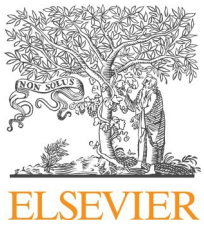

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# Development and characterization of ion-releasing fiber-reinforced flowable composite

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ABSTRACT

*Objective:* This study aimed to develop and characterize an ion-releasing experimental fiber-reinforced flowable composite (Bio-SFRC) and dentin treatment solution made of poly (acrylic acid) (PAA) with a high molecular weight. In addition we also evaluated the interface structure and mineralization potential between the Bio-SFRC and dentin.

*Methods:* Some mechanical properties (flexural properties and fracture toughness) of Bio-SFRC in comparison with commercial inert (G-aenial Flo X) and ion-releasing materials (ACTIVA-BioActive Base/Liner and Fuji II LC) were assessed (n = 8/group). Calcium-release at different time-points was measured during the first six weeks by using a calcium-ion selective electrode. Surface analysis of composites after being stored in simulated body fluid (SBF) was investigated by using SEM/EDS. Dentin disks (n = 50) were prepared from extracted sound teeth and demineralization was simulated by acid etching. SEM/EDS was used to evaluate the microstructure of dentin on the top surface and at interface with composites after being stored in SBF.

*Results:* Bio-SFRC showed higher fracture toughness (1.6 MPa $m^{1/2}$) ($p < 0.05$) compared to Flo X (1.2 MPa $m^{1/2}$), ACTIVA (1 MPa $m^{1/2}$) and Fuji II LC (0.8 MPa $m^{1/2}$). Accumulative calcium release after six weeks from Bio-SFRC (15 mg/l) was higher than other tested ion-releasing materials (≈ 6 mg/l). Mineralization was clearly seen at the interface between treated dentin and Bio-SFRC. None of the commercial tested materials showed signs of mineralization at the interface and dentinal tubules remained open.

**Significance.**

Developing such reinforced ion-releasing flowable composite and PAA solution might offer the potential for mineralization at the interface and inside the organic matrix of demineralized dentin.



## 1. Introduction

Restorative dentistry has slowly moved from biocompatibility to bioactivity in the past few years. Scientific interest in

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ion-releasing restorative materials is growing in parallel with the evolution of minimally invasive dentistry [1]. However, the term bioactive or biomineralizing materials is still a subject of controversy. While some biomaterial scientists believe that a bioactive material should be able to precipitate hydroxyapatite on its surface [1], others disagree [2]. Nevertheless, they should develop an active interface with biological tissue [1,3].

To treat dental caries via minimally invasive approaches, restorative materials should have buffering and mineralizing capabilities to arrest caries while keeping their stability over time and resistance to occlusal stress [4]. The goal of such approaches lie in reducing the excavation or drilling of dental tissues and instead supporting their healing and repair. As a result, dental materials scientists have been attempting to mineralize dentin under restorations in order to biomimetically restore intact dentin [1]. To have an ideal dentin mineralization, two things are needed. First, using chemically synthesized (dentin treatment solution) alternatives for specific dentin matrix proteins that are required for mineralization [5,6]. Second, an ion-releasing cavity liner or base to be used as a source for calcium and phosphate [7,8].

A recent bioengineering study on biomimetic mineralization [5] showed that poly(acrylic acid) (PAA) with specific molecular weight and concentration in amorphous calcium phosphate solution, facilitated collagen intrafibrillar mineralization via controlling the growth of hydroxyapatite crystals. This means that PAA could be a good dentin treatment solution and have a positive role in the formation and stabilization of amorphous calcium phosphate (ACP) during the dentin mineralization process [7].

To promote dentin remineralization and minimize caries, bioceramic particles such bioactive glass and calcium phosphate in different forms have been added to dental composites [9]. However, it is clear from the literature that contemporary bioactive or ion-releasing resin-based composites that are used as cavity liners or bases still demonstrate limitations due to their brittleness and low fracture toughness and other mechanical properties when used under large restorations [10–12]. As those non-silinaized bioactive particles do not have a reinforcing role such as silanized glass particles or fibers. In addition, dissolution of bioactive particles might also negatively affect the long-term mechanical stability of the composite [13]. On the other hand, flowable composites reinforced with short randomly oriented glass fibers showed improved fracture toughness, mechanical stability and ability to arrest crack propagation [14–16]. In fact, appropriate physical and mechanical properties and satisfactory ion-releasing are all characteristics that cavity liners should fulfill, especially for selectively excavated teeth and their restorations [17,18].

Developing materials that combine special interactive characteristics to meet the requirement for the dentin mineralization process is a challenging mission. To our best knowledge, in dentistry there is a lack of such a combined system. Thus, our research aim was to develop and characterize a novel system combining ion-releasing (bioactive) fiber-reinforced flowable composite (Bio-SFRC) and dentin treatment solution made of PAA with a specific molecule weight and concentration.

The hypotheses were (1) flowable ion-releasing composite (Bio-SFRC) could be reinforced by incorporating randomly distributed glass microfibers, (2) PAA solution can promote dentin remineralization underneath the composite material, (3) the adoption of such a system (Bio-SFRC and PAA) will demonstrate adequate response of mineralization at the interface with demineralized dentin.

## 2. Materials and methods

### 2.1. Materials

Bisphenol-A-glycidyl dimethacrylate (bis-GMA) was purchased from Esstech Inc. (Essington, PA, USA). Diurethane dimethacrylate (UDMA), hydroxyethyl methacrylate (HEMA), pentaerythritol tetraacrylate (PETA), camphoroquinone (CQ), N,N′-dimethyl aminoethyl methacrylate (DMAEMA) and poly (acrylic acid) (PAA) were obtained from Sigma-Aldrich Co. (St Louis, MO, USA). Borosilicate glasses containing $TiO_2$ and ZnO filler particles (Ø 0.7 μm) were received from Schott AG (UltraFine GM27884, Schott, Landshut, Germany). Carbonated apatite particles (Cytrans®, Ø 10 μm) were provided from GC Corporation (Tokyo, Japan). Calcium carbonate powder having a primary particle size of 200 nm was supplied by Shiraishi Central Laboratories (Hyogo, Japan). All of the reagents were used without purification.

Commercial inert flowable composite (G-aenial Flo X, GC Corporation) and ion-releasing materials (ACTIVA-BioActive Base/Liner, Pulpdent, Watertown, MA, USA and Fuji II LC, GC Corporation) were used as controls.

### 2.2. Production of bioactive fiber-reinforced flowable composite

Experimental bioactive flowable short fiber-reinforced composite (Bio-SFRC) was prepared by mixing 38 wt% of dimethacrylate based resin matrix (bisGMA/UDMA/HEMA/PETA = 30/23/30/17 with 0.7 wt% CQ and 0.7 wt% DMAEMA as a photoinitiator system) to 21 wt% of $TiO_2$ and ZnO containing borosilicate glass powder, 18 wt% of carbonated apatite particles, 3 wt% of calcium carbonate powder and 20 wt% of short fibers. The E-glass fibers (as-received silanized) having length scale of 200–300 micrometer (Ø6 μm), so-called microfibers and non-silanated powders were added gradually to the resin matrix. The mixing was carried out by using a high speed mixing machine for 2 min (Hauschild Speed Mixer DAC 400.1, 3500 rpm). Temperature change during the mixing were monitored using an infrared thermometer.

### 2.3. Preparation of dentin treatment solution

Dentin treatment solution was synthesized by mixing deionized distilled water and PAA with specific high molecular weight (450 kDa) and low concentration (10 mg/L). The PAA solution was stirred for 24 h at room temperature.

### 2.4. Mechanical tests of Bio-SFRC

Three-point bending test specimens (2 × 2 × 25 $mm^3$) were made from each experimental (Bio-SFRC) and commercial materials (Fuji II LC, ACTIVA, Flo X). Bar-shaped specimens were made in half-split stainless steel molds between transparent Mylar sheets. Polymerization of the materials was done using a hand light-curing unit (Elipar LED, TM S10, 3 M ESPE, Germany) for 20 s in five separate overlapping portions from both sides of the metal mold. The wavelength of the light was between 430 and 480 nm and average light irradiance was 1600 $mW/cm^2$ (MARC® Resin, BlueLight analytics Inc., Halifax, Canada). The specimens from each material (n = 8) were either stored dry (for one day/37 °C) or in SBF (simulated body fluid) for 30 days at 37 °C or boiled in deionized distilled water for 16 h before testing. The three-point bending test was conducted according to the ISO 4049 standard (test span: 20 mm, cross-head speed: 1 mm/min, indenter: 2 mm diameter). All specimens were loaded into a material testing machine (model LRX, Lloyd Instruments Ltd., Fareham, England) and the load-deflection curves were recorded with PC-computer software (Nexygen 4.0, Lloyd Instruments Ltd., Fareham, England). Flexural strength (FS) and flexural modulus (FM) were calculated from the following formula:

$FS = 3\ F_m I / (2bh^2)$ $FM = SI^3 / (4bh^3)$.

Where $F_m$ is the applied load (N) at the highest point of the load-deflection curve, I is the span length (20 mm), b is the width of the test specimens and h is the thickness of the test specimens. S is the stiffness (N/m) S=F/d and d is the deflection corresponding to load F at a point in the straight-line portion of the trace.

Single-edge-notched-beam (SENB) specimens (2.5 × 5 × 25 $mm^3$) according to an adapted ISO standard method (ASTMD5045–14; ISO/NP 13586) were prepared to determine fracture toughness [19,20]. A custom-made stainless steel split mold was used, which enabled the specimen's removal without force. An accurately designed slot was fabricated centrally in the mold extending until its mid-height, which enabled the central location of the notch and optimization of the crack length (x) to be half of the specimen's height. The material was inserted into the mold placed over a Mylar-strip-covered glass slide in one increment. Before polymerization a sharp and centrally located crack was produced by inserting a straight edged steel blade into the prefabricated slot. Polymerization of the material was carried out for 20 s in five separate overlapping portions. The upper side of the mold was covered with a Mylar strip and a glass slide from both sides of the blade, before being exposed to the polymerization light. Upon the removal from the mold, each specimen was polymerized also on the opposite side. The specimens from each group (n = 8) were stored dry at 37 °C for 24 h before testing. The specimens were tested in three-point bending mode, in a universal material testing machine at a crosshead speed of 1.0 mm/min. From the load values at fracture, fracture toughness ($K_{IC}$) was calculated through the following formula [21]:

$$K_{IC} = [P\ L/B\ W^{3/2}]\ f(x)$$

where: $f(x) = 3/2x^{1/2}\ [1.99\text{-}x(1\text{-}x)\ (2.15–3.93x+2.7x^2)]\ /\ (1+2)\ (1\text{-}x)^{3/2}$ and $0 < x < 1$ with x = a/W.

Here P is the maximum load in kilonewtons (kN), L is the span length (2 cm), B is the specimen thickness in centimeters (cm), W is the specimen width (depth) in cm, x is a geometrical function dependent on a/W and a is the crack length in cm.

### 2.5. Calcium ion release and surface microstructure characterization

Three disc-shaped specimens (15 mm in diameter and 1 mm in thickness) for each tested material were prepared in a metal mold. The material was packed into the mold and covered on both sides with Mylar strips and microscopic glass slides to extrude the excess material. Polymerization was performed according to manufacturer recommendation. All specimens were then polished to remove the oxygen inhibition layer and each specimen was immersed in a plastic receptacle containing 5 mL of deionized water at 37 °C. Each 5 mL of storage water was mixed with 0.1 mL of ionic strength adjustable buffer (ISA) solution and analyzed for calcium ions with the use of an ion-specific electrode (Orion Electrode, Orion Research Inc., Boston, MA, USA) connected to an ion analyzer supplied with the measuring unit. Measurements of calcium released were studied at 4 h, 1 day, 2 days, 7 days, 14 days, 3 weeks and 6 weeks. The solution was gently stirred during the analysis in a non-heated magnetic stirrer. The system was calibrated prior to each evaluation with calcium standards ranging from 0.1 to 200 ppm. The released ions were reported in cumulative concentrations.

Surface microstructure characterization of disc specimens (n = 3) made of tested materials after 2 weeks storage in SBF (under a shaking speed of 100 rpm) at 37 °C was analyzed using SEM and energy-dispersive spectroscopy (EDS).

### 2.6. Dentin disks preparation and experimental design

Fifty extracted, sound and caries-free wisdom teeth (acquired from the teaching clinic of the Institute of Dentistry, University of Turku) were selected. Upon collection, adhering soft tissues were removed under running water and the teeth were stored in a 0.5% chloramine T solution at 4 °C for a period not exceeding 2 months. Dentin disks (2 mm thick) were prepared a using ceramic cutting disc operating at a speed of 100 rpm (Struers, Glasgow, Scotland) under water cooling. Then, the upper surfaces of dentin disks were polished with an automatic grinding machine, 1200-grit, 300 rpm under running water (Struers Rotopol-11). To simulate demineralized dentin, disks were etched using 37% phosphoric acid (Scotchbond Universal Etchant, 3 M ESPE, USA) at room temperature for 20 s and rinsed carefully with distal distilled water for 1 min [7].

For testing the mineralization on the top surface of demineralized dentin, disks (n = 30) were either surface treated/rinsed with PAA solution (n = 15) or without (control, n = 15) before immersion in SBF prepared according to well known formula [22]. Disks were stored in SBF for 7 days

(steady) at 37 °C and solution was refreshed every day. Then, microscopic characterization of the dentin surface (with/without PAA solution) after different time points (1, 3 and 7 days) was performed.

For testing the interface between the tested materials and the demineralized dentin, Bio-SFRC and commercial materials (Fuji II LC, ACTIVA, Flo X) were applied on disks (n = 5/per material) and light-cured (Elipar LED) according to the manufacturer's instructions. Then, composite/dentin disks (n = 20) were immersed in SBF for 2 weeks (steady) at 37 °C. SBF solution was refreshed every day for 1 week. Disks were then broken into two halves for microscopic evaluation of the interface between the dentin and tested materials.

### 2.7. *Microscopic analysis*

SEM and EDS (GeminiSEM 450, Carl Zeiss & LEO, Oberkochen, Germany) provided the characterization of the microstructure of the investigated specimens. Specimens were stored in desiccator for one day. Then, they were coated with a gold layer using a sputter coater in a vacuum evaporator (BAL-TEC SCD 050 Sputter Coater, Balzers, Liechtenstein) before the SEM/EDS examination.

### 2.8. *Statistical analysis*

The data were statistically analyzed with SPSS version 23 (SPSS, IBM Corp.) using analysis of variance (ANOVA) at the $p < 0.05$ significance level, followed by a Tukey HSD post hoc test to determine the differences between the groups. Levene's test was used to test the normality of data.

## 3. Results

### 3.1. *Mechanical properties*

Levene's test revealed that the variances were homogenous and equal across groups. The mean values of flexural strength, flexural modulus and fracture toughness for tested experimental Bio-SFRC and commercial materials with standard deviations (SD) are summarized in Fig. 1. Two-way ANOVA demonstrated that both material type and aging condition had significant effect on the tested flexural properties ($p < 0.05$). All materials showed reduction in flexural strength after accelerated hydrothermal aging (boiling), between 15 % and 47 %, but only for ACTIVA and Bio-SFRC the reduction was considerable. Flo X presented the highest flexural strength (133 MPa) in dry condition among all tested materials, followed by Bio-SFRC (117.3 MPa) and ACTIVA (99.5 MPa). On the other hand, Fuji II LC had the lowest flexural strength values (40–59 MPa) before and after aging. ACTIVA had the lowest flexural modulus values before and after aging, while Bio-SFRC presented the highest in dry condition among the materials tested. According to the results, the SBF storage (30 days) has no significant impact on

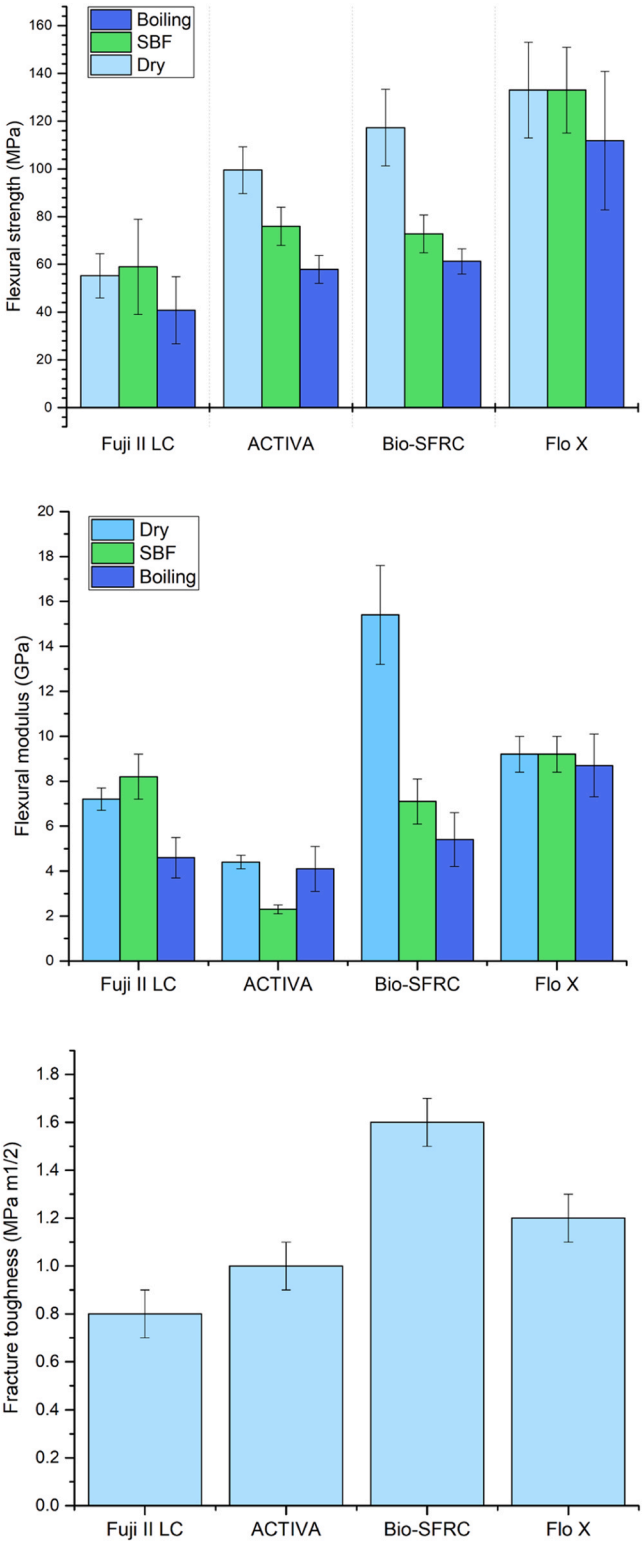


**Fig. 1 – Bar graph showing means flexural strength (MPa), flexural modulus (GPa) and fracture toughness ($K_{IC}$) with standard deviations (SD) of tested materials.**

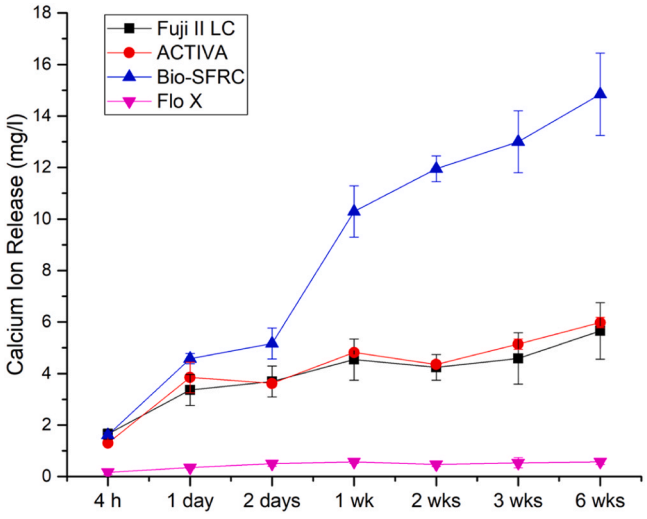


**Fig. 2 – Cumulative concentrations of the released calcium ion (mg/l) after 6 weeks of immersion in deionized water.**

the tested flexural properties of Fuji II LC and Flo X ($p > 0.05$).

Bio-SFRC had significantly higher fracture toughness (1.6 MPa $m^{1/2}$) ($p < 0.05$) compared to tested commercial materials. Fuji II LC presented the lowest fracture toughness (0.8 MPa $m^{1/2}$) which was significantly different ($p < 0.05$) from ACTIVA (1 MPa $m^{1/2}$) and Flo X (1.2 MPa $m^{1/2}$).

### 3.2. *Calcium ion release and surface characterization*

Fig. 2 presented the cumulative calcium release for the tested materials after a six-weeks storage period in deionized water. Ion analysis showed clear differences between the materials. The highest calcium release measurement was located for Bio-SFRC and lowest for Flo X among other materials. SEM/EDS surface examination of investigated materials after 2 weeks storage in SBF is presented in Fig. 3. SEM/EDS revealed typical surface microstructure of each tested material with various ingredient ratios. Major elemental composition determined with EDS is presented in Table 1. SEM/EDS analysis proposed a justification for dissimilar mineralization potential among tested materials. Bio-SFRC showed signs of mineralization on the surface (Fig. 3C), while none of the commercial tested materials showed any mineralization (Fig. 3A, B, D).

### 3.3. *Mineralization of demineralized dentin disks*

The effect of applying PAA treatment solution on demineralized dentin has been shown in Figs. 4 and 5. SEM images illustrated clearly that PAA solution promotes the mineralization process in the presence of calcium/phosphate rich media. Fig. 4 (B & C) showed a formation consisting of a thick mineralization layer (3–5 μm) on the demineralized dentin surface occluding all dentinal tubules after 7 days storage in SBF. On the other hand, lack of mineralization was seen with non-PAA treated dentin disks (control) and dentinal tubules remained open (Fig. 4A). After PAA solution treatment, the mineralization of the demineralized dentin disks started after 3 days in SBF and from deep inside the dentinal tubules (Fig. 5).

Fig. 6 showed the interface between demineralized dentin and representative examples for each of the tested materials after two weeks storage in SBF. None of the commercial tested materials showed signs of mineralization at the interface and dentinal tubules remained open, while Bio-SFRC showed signs of mineralization at the interface and inside dentinal tubules (Fig. 6A). Tag-like microstructures were clearly detectable in SEM images of the detached Bio-SFRC at the interface with treated dentin (Fig. 6B). An elemental mapping analysis showed that calcium in addition to silica and carbon were the major compositions of these tags. Furthermore, mineralization at the interface was detectable by the formation of a calcium rich layer (Fig. 7).

## 4. Discussion

Researchers have been trying for a long time to mineralize dentin under restorations by using different ion-releasing materials. At first, this is linked to the increased concern in minimally invasive approaches of caries management. On the other hand, the rising interest in the protective effect of mineralization on demineralized or etched dentin beneath restorations has prompted efforts to develop mineralizing techniques that restore the function and structure of minerals-depleted collagen fibrils [23,24]. Unfortunately, the current commercially ion-releasing materials are not able to immediately mineralize the residual caries-affected or etched dentin [10] and they are suffering from a lack of satisfactory mechanical properties and especially toughness when used as a dentin replacement material [10–12].

Considering the trade-off between mechanical properties and ion release. The outcomes of the present study approve the hypothesis that microfibers can still have a substantial impact on fracture toughness and flexural strength together with the easy to use properties of a high-flowable ion-releasing material. The experimental flowable Bio-SFRC showed enhanced resistance capability to crack propagation that is fracture toughness, which could be explained by the fiber and matrix related properties of the material. Despite the fact that microfibers in this flowable material are shorter than the critical fiber length. The aspect ratio is above 30, which offers reinforcement to the materials and thus, could efficiently accept the stress transferred from the matrix. In addition, the fibrous structure could offer a long-term stability for ion-releasing Bio-SFRC material [16].

In this investigation, the effect of SBF storage and hydrothermal accelerated aging on the materials' flexural strength was studied. In fact, this laboratory hydrothermal aging may not be directly translated into the clinical situation, but according to existing literature, it provides an indication of the long-term materials' stability [25,26]. Our data support the previous finding, which stated that boiling dental composite in water could induce quick penetration of water into the matrix of composite structure and as a consequence softening the composite matrix occurs [26]. Furthermore, the penetrated water was thought to contribute to the hydrolytic degradation of non-silinaized bioactive particles and induce

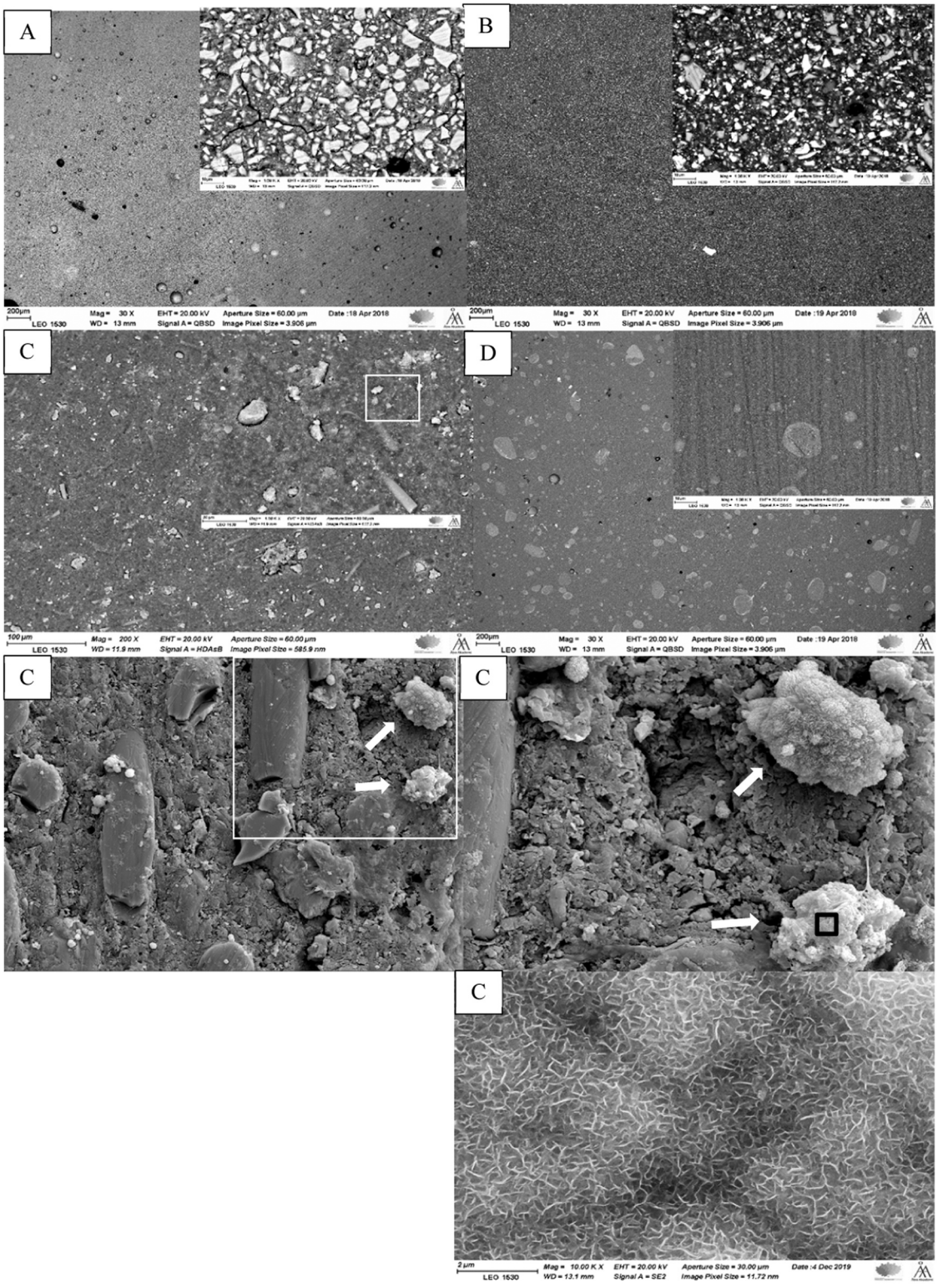


**Fig. 3 – SEM images at 30x & 1000x (upper right corner) magnifications of the surfaces of investigated materials after 2 weeks of immersion in SBF. (A) Fuji II LC; (B) ACTIVA; (C) Bio-SFRC; (D) Flo X. White arrows in the lower images (C) showed places of mineralization on Bio-SFRC surface with different (2kx, 5kx & 10kx) magnifications.**

hydrolysis of the particle-matrix interface, which resulted in increasing the resin phase softening [13]. Thereby, the reduction in the flexural properties of the tested materials after hydrothermal aging and SBF storage may be the results of these mechanisms. However, in this study the 30-days SBF storage period was not long enough to cause reduction in

**Table 1 – Major elemental compositions of investigated materials determined with EDS surface analysis after 2 weeks of storage in SBF.**

| Materials | Weight % |
|---|---|
| Fuji II LC | C 14.9, O 31.2, Na 0.7, F 9.7, Al 11, Si 9.5, Ca 1, Sr 22 |
| ACTIVA | C 35.5, O 35.3, Na 1.3, F 2, Al 3.3, Si 10.6, P 0.8, K 0.7, Ca 3.1, Ba 7.4 |
| Bio-SFRC | C 29.1, O 36.6, Na 2.8, Al 1.3, Si 9.7, P 4.3, K 1.3, Ca 12.4, Ti 0.9, Zn 1.6 |
| Flo X | C 27.5, O 41.8, Na 0.5, Al 3.3, Si 14, K 0.3, Ba 12.6 |

flexural strength for Flo X and Fuji II LC. It has been previously reported that exposure of glass ionomer materials to water improved the strength regardless of the presence or absence of resin reinforcement [27]. This was explained by the maturation of the cement matrix which became more rigid with time.

Different laboratory techniques (SEM, TEM, FTIR, XRD, EDS, EPMA, Micro-CT, hardness and nano-indentation) have been mentioned in the literature to identify the change in mineral content or mineralization of tooth substrate. Despite the significance of these techniques, they require careful sample preparation and their ability to detect clinically relevant mineralization may be limited. In this study, we examined the mineralization formation on the surface of human dentin and at interfaces between ion-releasing materials and dentin, which may be more informative and representative.

Our results support using PAA with specific high molecular weight and low concentration as a dentin treatment solution to promote mineralization. Therefore, the second hypothesis was accepted. We demonstrated that after application of PPA, a layer of mineralization was formed on the demineralized dentin surface occluding opened dentinal tubules after a few days of storage in SBF (Fig. 4B). Interestingly the PAA application promoted a fast acting mineralization process deeply inside the dentinal tubules (Fig. 5). We speculated that the used PAA has a high negative charge, which provides its ability to attract ions and stabilize the growth of ACP around dentinal collagen fibers. This is in line with previous in-vitro studies where PAA-ACP solutions showed stability and controlled growth of PAA-ACP agglomerates in the presence of collagen matrix [5,7,28]. Our results support the finding of Qi et al., who showed that solution containing a very low concentration of PAA with high molecular weight, minerals quickly aggregated, crystallized and precipitated [5]. Authors emphasized the potential of using this PAA-ACP solution to manufacture scaffolds and constructs for tissue engineering with exceptional properties that match those of bone [5].

Studies from existing literature had offered evidence that minerals within collagen fibers start as stable amorphous mineral particles that eventually transform into hydroxyapatite crystallites [29–31]. Gaining full thickness mineralization, would require this process to be diffuse and faster. This could be accomplished by using biomimetic analogs or a

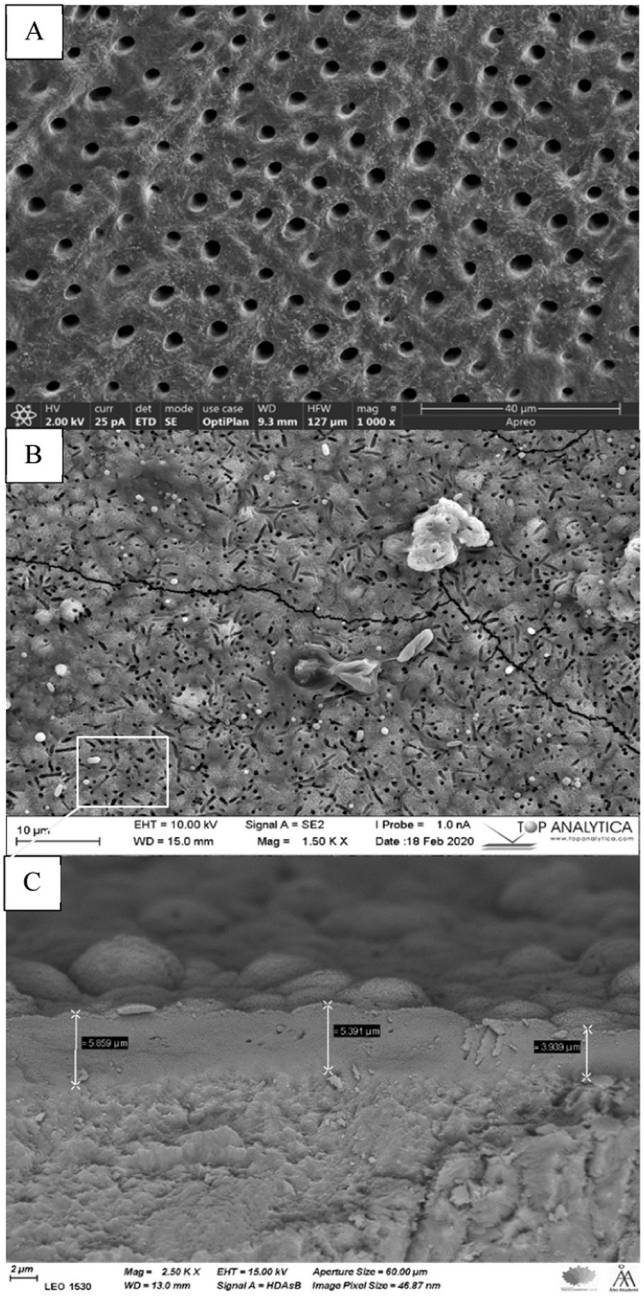


**Fig. 4 – SEM images (1000×, surface view) of demineralized dentin disks without (A, control) or with (B) PPA treatment solution after one week storage in simulated body fluid. Cross sectional view (C) at 2500× magnification showing the thickness of mineralized layer (3–5 µm).**

dentin treatment solution [32], which replaces the action of non-collagenous matrix proteins that are usually involved in the typical mineralization process. According to Tay and Pashley, these synthetic alternatives are thought to provide scattered nucleation sites, allowing metastable crystals to form in the gap zone between collagen fibrils [23]. Thereby protecting crystals from further hydrolysis and repair their functional properties. However, further investigation of the mineralization process is still needed.

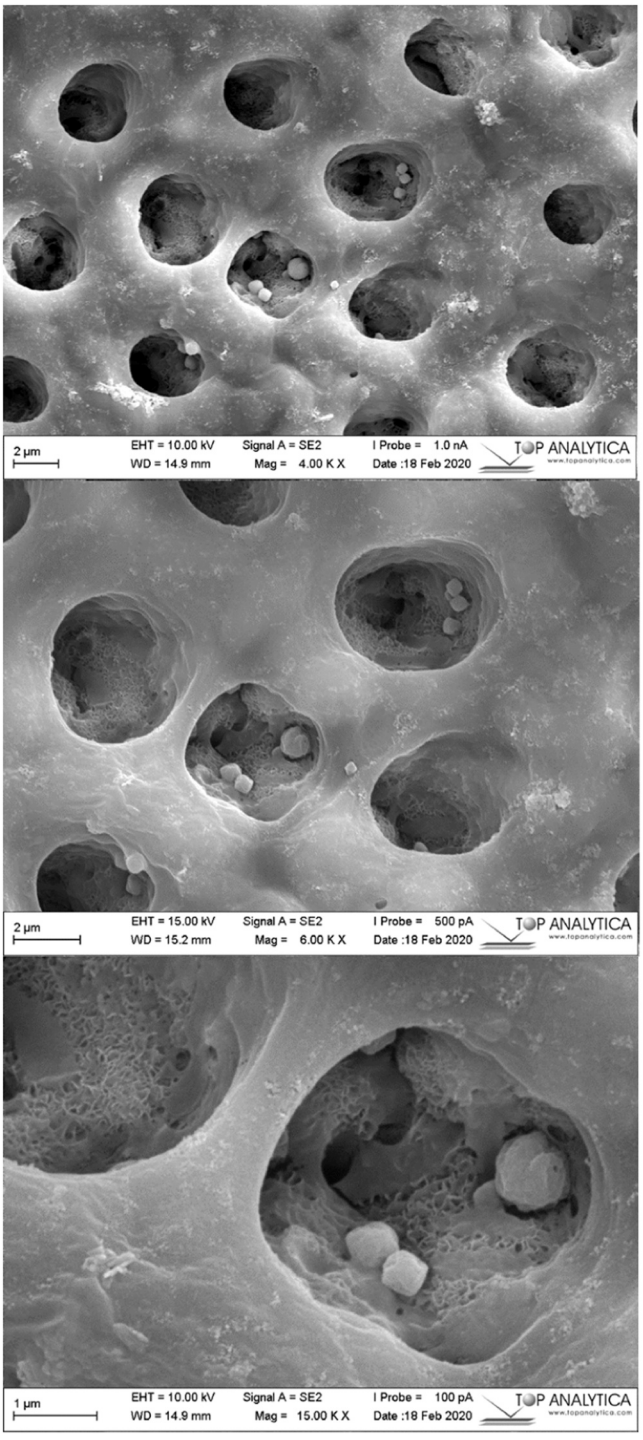


**Fig. 5 – SEM images with different magnifications (4kx, 6kx & 15kx) of demineralized dentin disc treated with PAA solution and stored 3 days in SBF showing the mineralization inside the dentinal tubules.**

In the SEM images, a few microns wide band or layer of mineralized structure was visible at the interface between Bio-SFRC and treated dentin specimens (Fig. 6 & 7) but not in the Fuji II LC, ACTIVA, and Flo X specimens (Fig. 6). A calcium rich layer was formed on the surface exposed to the Bio-SFRC (Fig. 7) and the dentinal tubules appeared to be blocked and did not open to the surface unlike the tubules of other specimens. This could be attributed to the presence Bio-SFRC as the source of ions and PAA as a dentin treatment solution to attract ions and stabilize the growth of ACP. Therefore, the third hypothesis was also accepted.

Formation of an interfacial layer between the demineralized dentin and ion-releasing materials like calcium silicate cements was previously reported by many authors [33–35]. They suggested that this layer forms in between the two substrates due to hydroxyapatite formation, although, our study showed that mineralization could be formed within the structure of the dentin as well. However, no direct comparison should be made as the materials, testing technique and samples in our study were different from the aforementioned studies. The flowable consistency of the experimental Bio-SFRC may have facilitated in its penetration through the opened dentinal tubules, where eventually overtime some crystalline clusters may form (Fig. 6). This is in accordance with Atmeh et al., and Reyes-Carmona et al., studies, which showed tag-like structure between calcium silicate cements and dentin [33,35].

It was not surprising that Flo X did not show any sign of mineralizing potential as this composite contains mainly barium glass fillers and it is not considered as an ion-releasing material. However, the tested commercial ion-releasing materials did not either show any mineralization potential even though they released a relative amount of calcium (Fig. 2). The potential mineralizing effects of materials like Fuji II LC and ACTIVA on demineralized or carious tooth tissues have been widely studied [36–41]. This has led to their use as part of minimally invasive methods for mineralizing caries-affected dentin. The mineralizing effect was principally attributed to the rich fluoride release by the glass ionomer like materials, which induces the formation of highly acid resistant fluoroapatites [42]. Ion exchange with dentin, including calcium and strontium, was also reported [43], and therefore indicated the dentin mineralization [36]. However, the role of glass ionomer-like materials in dentin mineralization remains controversial in the absence of conclusive results about this role [44]. Our results are in line with many laboratory studies, which could not demonstrate the mineralizing potential of glass ionomer like materials in demineralized dentin [37,45,46].

The amount of calcium released from Bio-SFRC after one and six weeks was more than double that of other tested ion-releasing materials (Fig. 2). This could be explained by the high concentration of functional bioactive fillers (> 20 wt%), in particular calcium carbonate and carbonated apatite (Cytrans) within the composition of Bio-SFRC [47–49]. The HEMA present in Bio-SFRC slowly absorbs water to allow for diffusion of ions and not being trapped within the polymeric matrix [11]. In addition, a recent report suggested that exposed short fibers might have a positive role in ion leaching from the cross-linked composite structure [50].

It was interesting to find that Flo X released little measurable amounts of calcium although it is a stable and not ion-releasing composite. Material Safety Data Sheets are usually incomplete and sometimes misleading [51]. In these sheets, the manufacturers are committed to give information

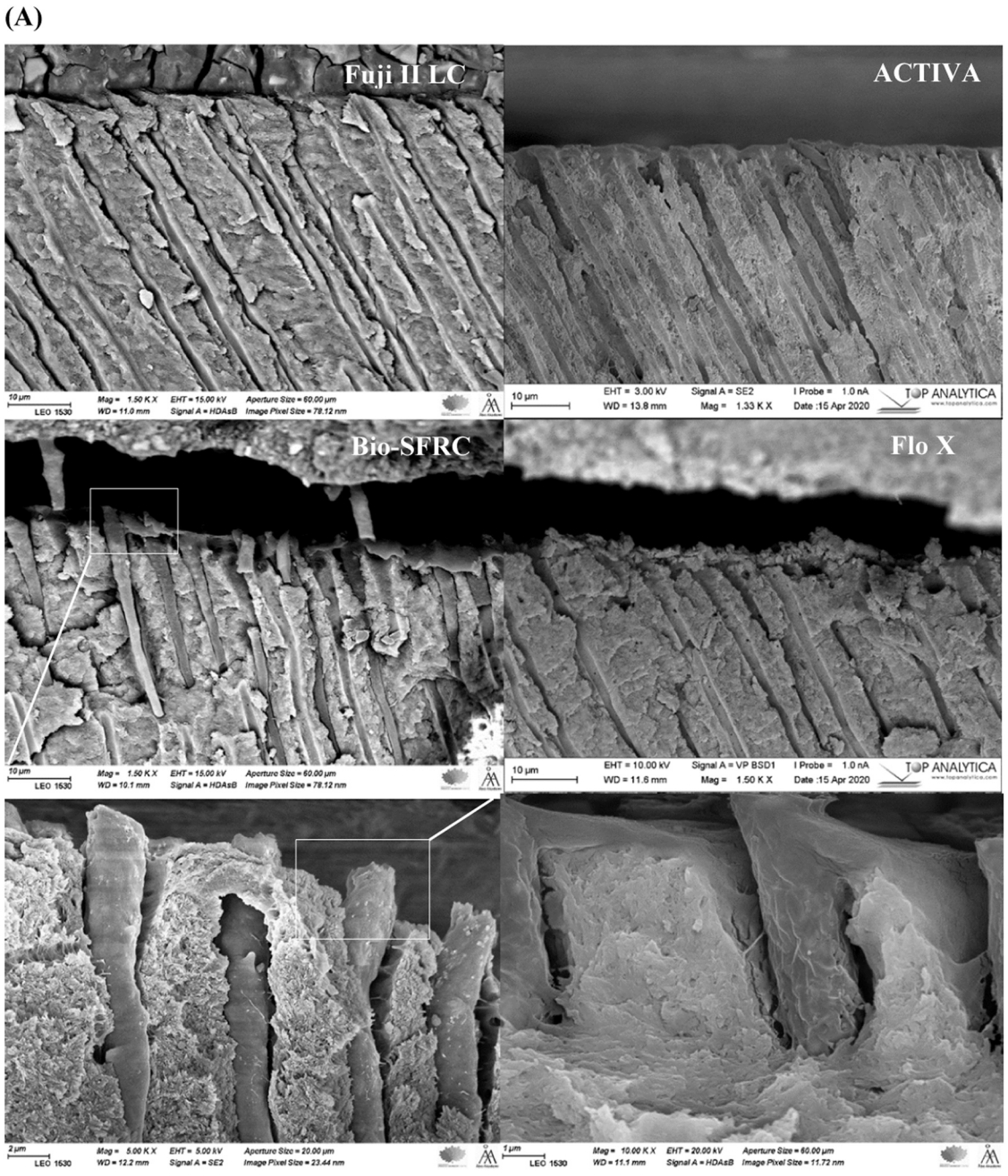


**Fig. 6 – (A) SEM images of sectioned demineralized dentin disks which were exposed to tested materials for 2 weeks in SBF. Bio-SFRC showed signs of mineralization at interface and inside dentinal tubules. (B) Tag-like structures with relatively large amounts of spherical shaped precipitate were detected on the detached Bio-SFRC at the interface. An elemental mapping analysis confirming the presence of the Ca rich structure.**

about the main ingredients (≥1 %). Meanwhile, most additives and some fillers and monomers are added in concentrations below 1 %. This might explain why Flo X released little calcium though calcium was not mentioned in the composition by manufacturers. It's important to highlight that the use of an ion-specific electrode to measure the calcium release is easy and quick to perform. However, the main disadvantage of the ISE is that it is only selective and not specific for calcium, which might cause some measurement error in the presence of interferences.

In the methodology, deionized water was used as a specimen storage solution because it is easily obtainable and easy to measure calcium in deionized water than in artificial saliva or SBF solutions. Therefore, the amount of calcium released cannot be expected to be released from the specimens at the same content as occurred inside one's mouth. It was not possible to see clearly the mineralization layer on the polished surface of tested materials after two weeks of storage in SBF (Fig. 3). Although Bio-SFRC showed signs of some mineralization in the form of needle-like apatite structures

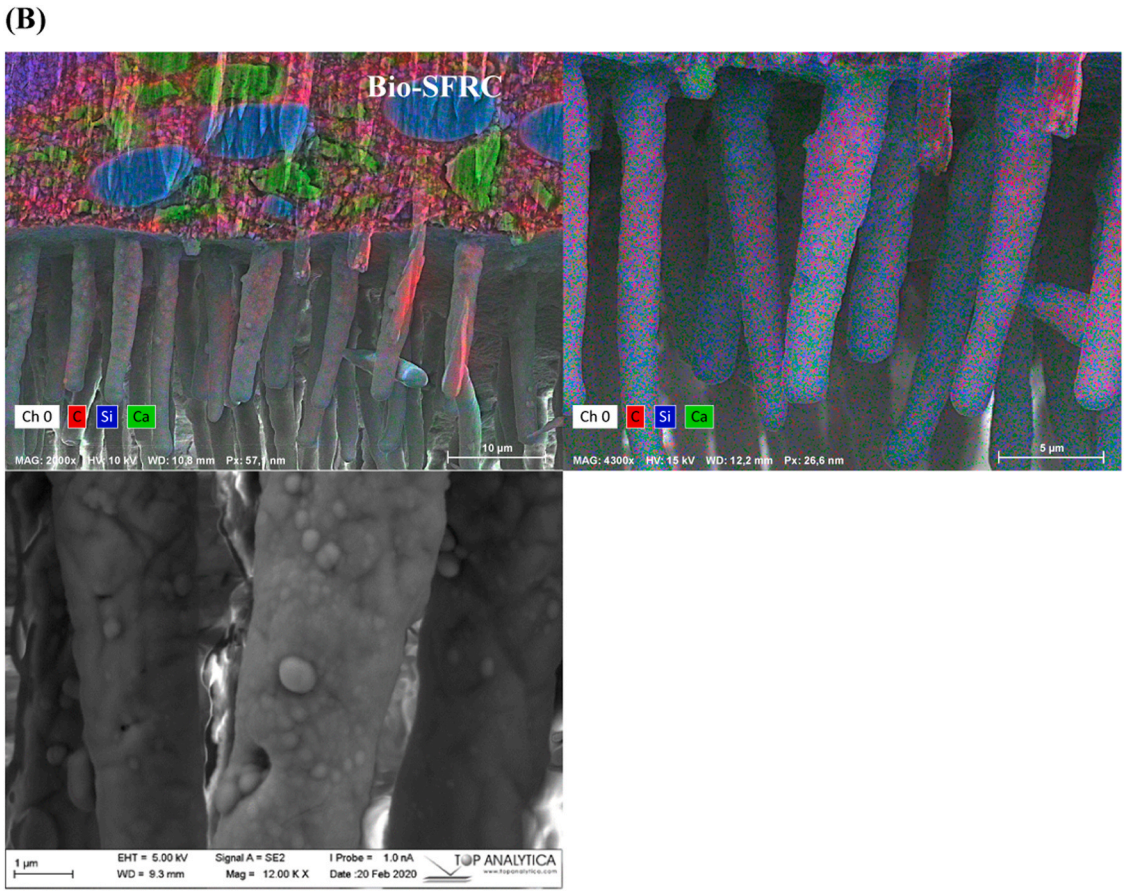


**Fig. 6 – (*continued*)**

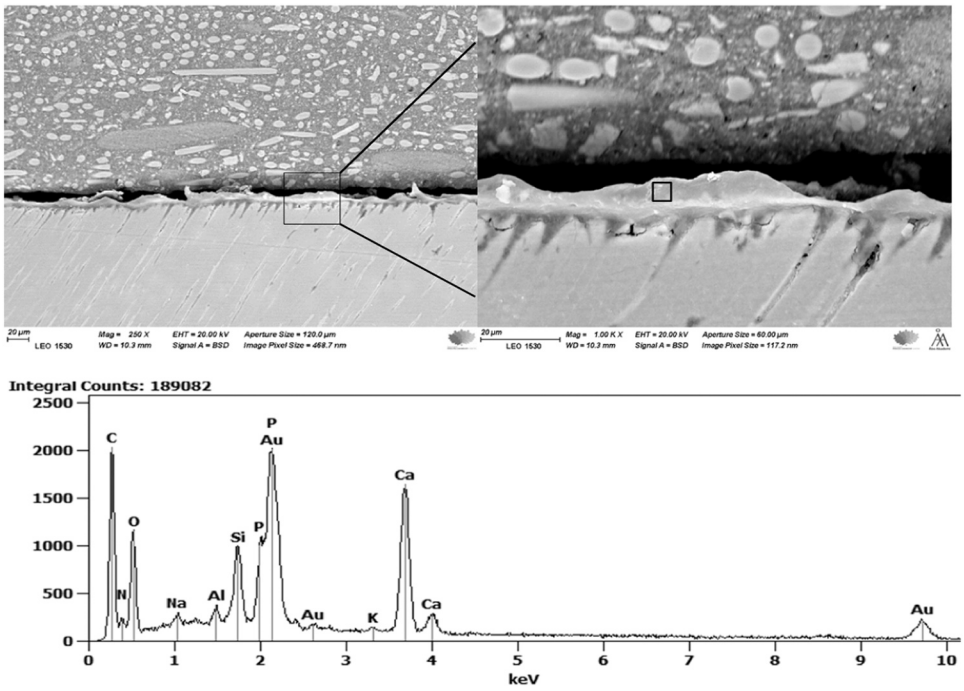


**Fig. 7 – SEM/EDS images with different magnifications (250× & 1000×) showed mineralization formation at the interface between activated dentin and Bio-SFRC after 2 weeks of storage in SBF.**

(Fig. 3C). Two weeks in SBF may be not enough immersion time to form a thick mineralized layer on composite surfaces as proven in Tiskaya et al., study [38]. In particular with Bio-SFRC, which demonstrated gradual elevation of accumulative calcium release, while other tested ion-releasing materials' accumulative calcium levels remain relatively constant after one week (Fig. 2). This was attributed to the resorption and release rate of the functional bioactive particles within Bio-SFRC structure. Calcium carbonate showed very fast resorption and release rates in comparison with other bioactive particles [49], while carbonated apatite showed a low resorption rate with a constant/prolonged release of calcium ions [47,48]. In accordance with previous reports [38,41] the amount of calcium release from Fuji II LC and ACTIVA may be too low to induce formation of any mineralization on the surface.

In addition to calcium and phosphorus oxides, zinc and titanium oxides were detected on the surface composition of Bio-SFRC (Table 1). Which might have inhibitory effects on biofilm formation and adhesion [52]. However, this issue will be investigated further in the near future.

This research was linked with certain difficulties and challenges associated with the preparation of the materials (PAA and Bio-SFRC) and the sensitivity of specimens' fabrication and cutting technique, As a result, it was necessary to develop a number of pilot studies to determine the most suitable protocols/compositions that could enable us to effectively use these techniques in studying this new interface. Further research is needed and an assessment of optimizing the formulation of these experimental materials is now in progress.

## 5. Conclusion

The use of Bio-SFRC in combination with PAA solution exhibited tougher and higher potential to induce mineralization in demineralized/etched dentin in comparison with the commercial ion-releasing materials.

## Declarations of Competing Interest

Author PV declares that he is a consultant for Stick Tech – Member of GC in training and research and development. Other authors do not have conflicts of interests.

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