# Grit blasted aggregates of hydroxyl apatite functionalized calcium carbonate in occluding dentinal tubules

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Hypersensitiivisellä dentiinillä tarkoitetaan tilaa, jossa potilas kokee yhtäkkistä viiltävää kipua, joka syntyy lämpötilanvaihtelun, kosketuksen, osmoottisen tai kemikaalisen ärsytyksen seurauksena. Oireiden taustalla on paljastunut dentiini- tai juurenpinta sekä avonaiset dentiinitubulukset. Nämä välittävät ärsykkeitä pulpan hermosoluille, mikä johtaa kipuaistimuksen syntyyn. Tässä *in vitro* -tutkimuksessa selvitettiin hydroksiapatiitilla päällystetyn kalsiumkarbonaattipartikkeleiden (FCC) kykyä tukkia avoimia dentiinitubuluksia ja saada aikaan biomineralisaatiota.

Poistetuista viisaudenhampaista valmistettiin 1 mm paksuja dentiinikiekkoja. Tutkimuksessa vertailtiin kolmea ryhmää: hiottu dentiini, EDTA –käsitelty dentiini ja FCC -hiekkapuhallettu dentiini. Puolet näytteistä upotettiin simulated body fluid (SBF) -nesteeseen mineralisaation tutkimiseksi. Dentiinitubulusten tukkiutumista ja dentiinipinnan mineralisaatiota arvioitiin elektronimikroskoopin avulla. Mineralisaatiopinnan alkuaineiden määritys suoritettiin energy-dispersive X-ray spectroscopy (EDX) -analyysin avulla. Mineralisaatiopinnan kiderakenne arviointiin X-ray diffraction (XRD) -analyysillä.

Tutkimuksessa kävi ilmi, että hiekkapuhalletut FCC -partikkelit tukkivat dentiinitubuluksia. EDTA -ryhmän näytteissä oli enemmän avoimia tubuluksia kuin FCC -ryhmän ja avoimet tubulukset olivat kooltaan suurempia. Ryhmät eivät kuitenkaan poikenneet mineralisaatiossa. Dentiinitubulusten tukkeutuminen FCC -partikkeleilla perustuu ensisijaisesti alkuperäisen partikkelin hajoamiseen ja partikkelien osien keräytymiseen tubulusten pinnalle. FCC:n mineralisaatiokyvyn todistamiseksi tarvitaan jatkotutkimuksia.

Asiasanat: hypersensitiivinen dentiini, hiekkapuhallus, kalsiumkarbonaatti, hydroksiapatiitti

# Sisällys

# Grit blasted aggregates of hydroxyl apatite functionalized calcium carbonate in occluding dentinal tubules

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# ABSTRACT

**Objectives:** This study aimed to investigate the effects of using hydroxyl apatite functionalized calcium carbonate (FCC) particles on occluding dentinal tubules.

**Methods:** Dentine specimens extracted from eighteen human molars with exposed dentinal tubules were divided into three groups (n=6/group): a) Cut surface with smear layer; b) EDTA (smear layer removed with 17% EDTA for 1 min); and c) Grit blasted functionalized calcium carbonate (FCC) with and air pressure of 280 kPa. Microscopic dentinal tubule occlusion, tubule diameter and tubule area were evaluated using scanning electron microscopy (SEM) before and after grit blasting. Biomineralization of specimens was carried out in a simulated body fluid (SBF). Elemental analysis of occluding materials was carried out using energy-dispersive X-ray spectroscopy (EDX). X-ray diffraction (XRD) analysis was performed to demonstrate the crystal structure of the biomineralized layer on dentine.

**Results:** FCC particles showed penetration into the dentinal tubules by breakage of their original particle shape and size. EDTA treated surface had higher number and larger size tubules than those with smear layer or grit blasted ($p<0.005$). SEM-EDX analysis revealed mineral precipitation of calcium phosphate on the SBF immersed dentin specimens. XRD analysis showed typical crystal structure of hydroxyl apatite for the biomineralized surface layer on dentine.

**Conclusions:** Grit blasted FCC particles initially occluded effectively the opened dentinal tubules and biomineralization occurred in tubules primarily occluded by the FCC particles. However, in the optimal in vitro conditions in SBF, no difference between biomineralization was found between the grit blasted surface and the control surface.

**Clinical significance:** Several materials and methods have been established for treatment of dentinal hypersensitivity although a golden standard treatment has not been discovered. Grit blasted functionalized calcium carbonate has a potential to occlude and remineralize exposed dentinal tubules. This could offer a more biological approach on treatment of dentin hypersensitivity.

# 1. INTRODUCTION

Dentine hypersensitivity (DH) is a frequent dental complaint that is mainly derived from the exposure of dentine due to enamel loss and/or gingival recession and is characterized by sudden short sharp pain[1]. The pain sensation is aroused by a stimulus of thermal, tactile, osmotic, evaporative or chemical change, which does not result from any other form of defect[2]. Several theories, such as the odontoblastic transduction theory, neural theory, pain gate control theory and Brannstrom´s hydrodynamic mechanism have been reported to be behind DH[3][4][5][6].

The origin of dentine exposure at the teeth' cervix or root is often seen as a consequence of periodontal treatment, the use of abrasive dentifrices, improper tooth brushing, and the consumption of acidic food and beverages that can be conductive to the loss of tooth structure[7][8]. Although a vast number of studies into the epidemiology of DH have been conducted, there is no consensus of its generality. To date, the prevalence rates of DH vary from 3 to 98%[8]. This wide range in prevalence may be attributed to the different methods used for diagnosing and testing dentine hypersensitivity[9]. A systematic review shows that the prevalence for DH following non-surgical periodontal treatment ranged from 63 to 90% one day after the treatment to 53 to 55% after one week[10]. Similar findings are reported in another systematic review where tooth sensitivity was recorded as high immediately after the majority of periodontal surgical procedures, which decreased naturally over time[11].

Dentine hypersensitivity is an enduring problem in dentistry where an effective treatment has not been discovered[7]. Dentinal tubules are filled with a transudate of pulpal fluid, which fluctuates in response to intense hot or cold stimuli. In cases where the enamel or cementum peripheral seals of dentine are broken, some dentinal fluid can leak out of the dentine[12]. In the process of removing calculus from root surfaces using curettes, a fine layer of cementum is also removed leaving smear layer-like debris on top of the exposed dentine. Dentinal fluid has the ability to gradually permeate across smear layer. The smear layer prevents dentine sensitivity due to its debris that are forced into each dentinal tubule during the creation of the smear layer that seals the tubules[13]. However, those smear layers promptly become colonized with bacteria, which forms biofilms that solubilize the

smear layer in 7-10 days[14][15]. Once the smear layer is lost, dentine tubules become more conductive, therefore patients experience dentine sensitivity[14].

If sensitive dentine is covered with plaque, a microbial diffusion into the pulp can take place, which provokes a mild inflammatory response. This can convert a sensitive dentine into a hypersensitive one[16]. *In vitro* dentine permeability tests have been used to investigate the effect of therapeutic agents for sensitive teeth. Many desensitizing therapies aim to occlude dentinal tubules reducing dentine permeability[17][18][19]. Fluorides and oxalates have been used for chemical occlusion[20] as well as Nd-YAG laser treatment[21]. Oxalate compounds have shown the ability to decrease fluid movements through dentine sections[22]. A study showed the capacity of oxalate solutions used in dentine to suppress the responses of the intradental nerves to hydrodynamic stimuli[23]. Other mechanisms that have been used to reduce dentine permeability include those that generate amorphous calcium phosphate in the tubules[24]. Cationic particles that bind electrostatically to anionic proteins on the walls of the tubules have been also used as a possible treatment for dentine sensitivity, as well as monomer resin systems for cross-linked polymers[25].

Dentine surface allows biomineralization to occur in the water containing liquid environment with excess silica, calcium and phosphorus. The mineral precipitation is calcium phosphates of various elemental ratios. The sources for the excess of mineralizing ions can be bioactive glasses[26] but tricalcium silicates have also been tested [27]. In optimal microenvironment where there is a presence of silica source, the precipitated amorphous calcium phosphate can form crystallized hydroxyl apatite, a process called biomineralization. *In vitro* biomineralization is believed to mimic the formation of a hydroxyl apatite (Hap) or carbonated apatite (CAp) layer *in vivo*. Crystallized hydroxyl apatite is more resistant to dissolution and therefore it may offer longer-lasting treatment outcomes for DH than amorphous calcium phosphates. *In vitro*, the biomineralization can be studied by immersing the material sample in a simulated body fluid (SBF), which is a modified calcium phosphate solution as reported in the literature[28]. Precipitation and crystal formation from a supersaturated solution of the constituent ions requires local, i.e. microenvironmental fluctuations in concentration of the interacting ions [29].

Surface modification by coatings of e.g. sol-gel processed bioactive glass or by tribochemical coating that includes grit blasting the dentine surface with particles of bioactive glass is known to promote the biomineralization of the dentine *in vitro*[30]. A new composition for the particles which could be used in tribochemical conditioning of dentine surface is calcium carbonate in a functionalized form with hydroxyl apatite. Therefore, the aim of this study was to investigate microscopically the effect of functionalized calcium carbonate (FCC) aggregates on the occlusion of dentinal tubules immediately after tribochemical grit blasting and after biomineralization in SBF.

# 2. MATERIALS AND METHODS

## 2.1 Specimen preparation

Eighteen intact extracted human molars were selected. The protocols used followed the ethical regulations of *Act on the Medical Use of Human Organs, Tissues and Cells* Section 20 (689/2012). Functionalized calcium carbonate (FCC) particles were obtained from Omya International AG (Oftringen, Switzerland). The FCC particles were characterized by their manufacturer to be 85% of hydroxyl apatite (Hap) and 15% of calcium carbonate (CC) with median particulate size (d50%) of 5.5µm and specific surface area of 110 $m^2/g$. FCC consists of co-crystallized calcium carbonate and calcium phosphate. It is characterized by a high porosity of 60% (v/v), and its surface shows a porous meshwork with a lamellar surface structure, which drives to a high specific surface area in the range of 70$m^2/g$ and represents the functional part of the particles[31]. The exterior surface of these particles shows the debris of the lamellae, while the inner part is composed of smaller pores with an average diameter of approx. 100nm. The inner porous structure shows high mechanical stability at intense compression pressures up to 200 MPa[32]. A SEM image of the FCC particles is shown in Figure 1.

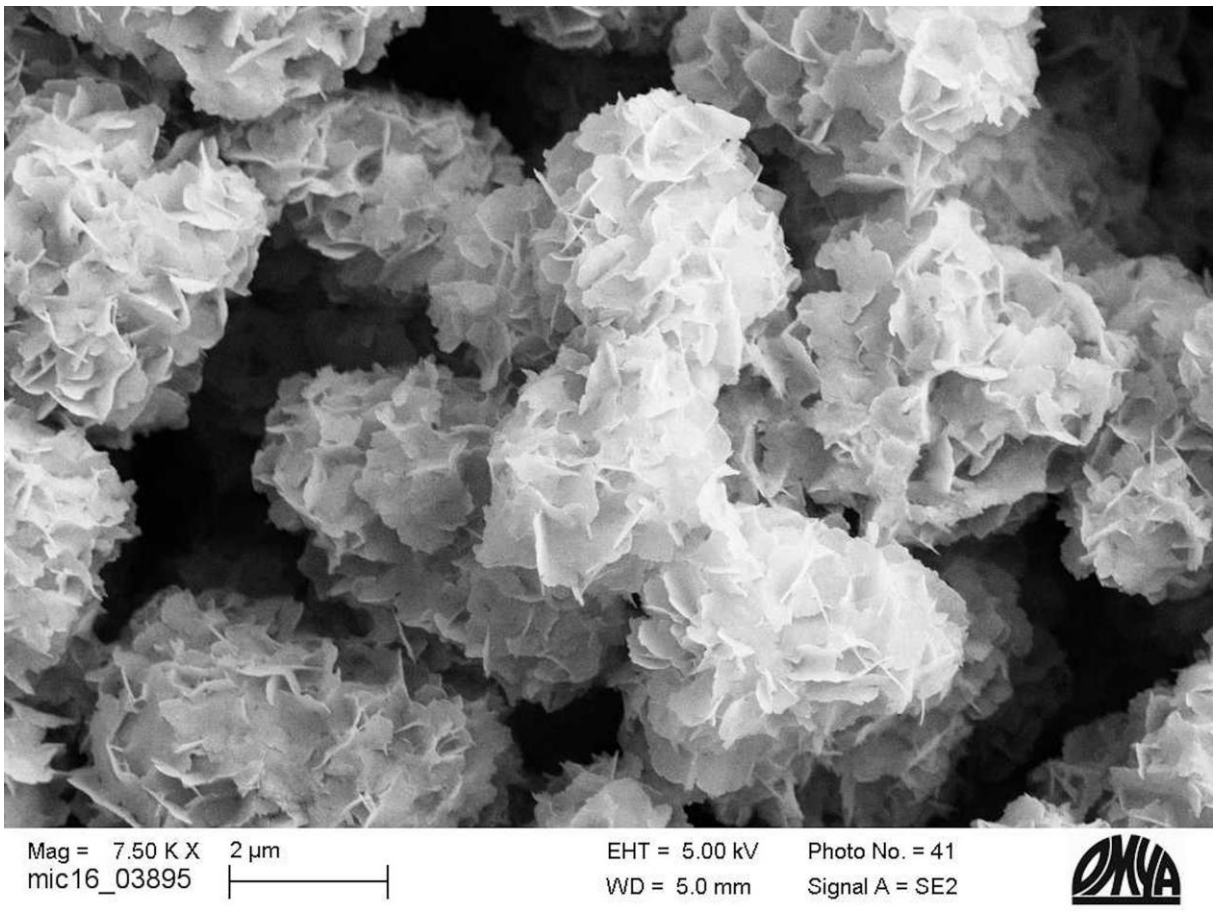


**Fig. 1** Scanning electron microscopy (SEM) image of functionalized calcium carbonate particles. Original magnification ×7500, bar=2µm

Teeth were divided into three groups: termed cut surface with smear layer, EDTA treated and FCC grit blasted treated. Each tooth was wet ground on two sides (buccally and lingually) with 300-grit (FEPA) silicon carbide paper under running water until dentine was reached. Subsequently, the teeth were cut into two pieces using a low-speed water-cooled diamond saw (Struers secotom 50) parallel to the long axis of teeth away from the pulp chamber. Dentine surfaces were polished with 500-grit silicon carbide paper under running water for 30 seconds to create a standardized smear layer. The smear layer was subsequently removed by treating the dentine surface in groups EDTA and FCC with 17% EDTA solution for 1 minute. The specimens were gently rinsed afterwards with distilled water for 10 seconds. FCC group was additionally grit blasted using an air-polishing device (Prophyflex 3, KaVo, Germany) with FCC particles. The treatment time was 10 seconds and the working air pressure of the air-polishing device was set to 280 kPa. The distance between the outflow opening of the handpiece and the dentine surface was kept constant

at 10 mm, and the treatment angulation was adjusted to 90 degrees. Excess FCC particles were removed using air blow for 2 seconds. For the biomineralization test, an additional group of specimens were made by coating the EDTA treated dentin surface with a layer of light curing resin composite to initially seal the dentinal tubules using AUDMA, UDMA and 1,12-dodecane-DMA based composite (Filtek™ Bulk Fill, 3M ESPE, St. Paul, MN, USA). Specimens of all groups were autoclaved in 121°C as a preparation for the biomineralization test.

## 2.2 Assessment of morphology and tubular occlusion

Scanning electron microscopy (SEM) using Phenom ProX (Phenom-World, Eindhoven, The Netherlands) operating at 15 kV was used in combination with digital image analysis (Phenom-World, PoroMetric) of the photomicrographs at ×6000 magnification to provide both qualitative and quantitative assessment, respectively. The mean tubule count, tubule diameter (μm) and area were calculated for each specimen. Three areas were randomly selected and analyzed from each specimen. The software automatically selected open tubules and provided tubule count, diameter and area of each tubule.

## 2.3 In vitro incubation of teeth in SBF solution

Half of the specimens from each group (n=6) were immersed in a simulated body fluid (SBF) for one week. SBF consisted of 136.8 mM NaCl, 4.2 mM $NaHCO_3$, 3.0 mM KCl, 1.0 mM $K_2HPO_4 \cdot 3H_2O$, 1.5 mM $MgCl_2 \cdot 6H_2O$, 40 mM HCl, 2.5 mM $CaCl_2$, 0.5 mM $Na_2SO_4$, 50 mM $(CH_2OH)_3CNH_2$; its pH was adjusted to 7.4. Dentine discs with composite coating (3M Filtek One Bulk Fill Restorative) were used as a negative control for biomineralization. Incubation was performed at 37°C for 7 days.

## 2.4 Microscopic analyses and imaging

The biomineralized dentine specimens of EDTA and grit blasted groups were analyzed using SEM. The analysis was made perpendicular to the mineralized dentine surface and on the fractured surface along the direction of dentine tubules. The specimens were air dried at room temperature (20°C ± 1°C) for 24 hours before being coated. Non-SBF specimens were sputter coated with gold for morphological analysis using SEM imaging. SBF immersed

specimens were sputter coated with carbon for energy dispersive X-ray spectroscopy (EDX). All specimens were stored in a desiccator for 24 hours prior imaging and analysis. Mineralized dentinal tubules were visually selected and measured using the SEM´s image analyzer. Three areas were randomly selected and analyzed from each specimen to assess dentinal tubule occlusion between the EDTA and grit blasted FCC specimens.

### 2.5 X-ray diffraction characterization

X-ray diffraction (XRD) characterization was performed for examining the crystallographic structure of the biomineralized layer with an instrument (Bruker D8 Discover) equipped with a Cu $K_\alpha$ x-ray source and scintillator point detector. The analysis was executed on the surface of FCC grit blasted and EDTA specimens. The material inside the tubules was not analyzed. The specimens were measured in the 2 theta range 20-60°, increment 0.04°, and 20 s per point.

### 2.6 Statistical analysis

Statistical analysis was performed using SAS JMP 10. Differences of dentinal tubule occlusion between the groups were analyzed using nonparametric comparisons for each pair using Wilcoxon rank sum test. *P*-values less than 0.05 were considered statistically significant.

## 3. RESULTS

### 3.1 Occluding dentinal tubules analysis

Functionalized calcium carbonate (FCC) grit blasted group showed tubular occlusion by breakage of particles' original shape and size. The percentage of occluded tubules in FCC treated samples was statistically significantly increased compared with the EDTA treated samples ($p<0.005$). Open tubule area and circle equivalent diameter were also statistically significantly decreased in FCC grit blasted samples compared with the EDTA treated ones ($p<0.005$). Characterized parameters of dentine surface are summarized in Table 1. Tubular occlusion is shown in Figure 2.

**Table 1.** Characterized parameters of dentine surface

| | Smear layer | EDTA | FCC |
|---|---|---|---|
| Percentage of occluded tubules | 70.7 (26.2) % | 4.7 (5.6) % | 85.4 (12.6) % |
| Circle equivalent diameter | 0.88 μm (0.37) | 1.51 μm (0.39) | 0.94 μm (0.42) |
| Open tubule area | 0.72 $\mu^2$ (0.63) | 1.92$m^2$ (0.90) | 0.84 $\mu m^2$ (0.77) |

(SD)

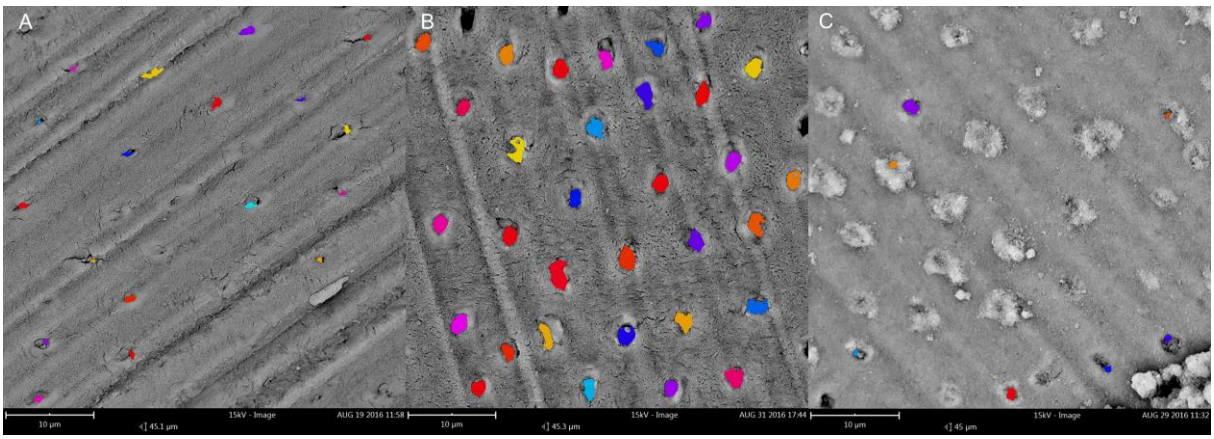


**Fig. 2** Scanning electron microscopy (SEM) images of dentine surfaces and porometric analysis of A. cut surface with smear layer; B. EDTA treated; C. FCC grit blasted samples. Colored areas represent the open tubules. Original magnification ×6000, bar = 10 μm.

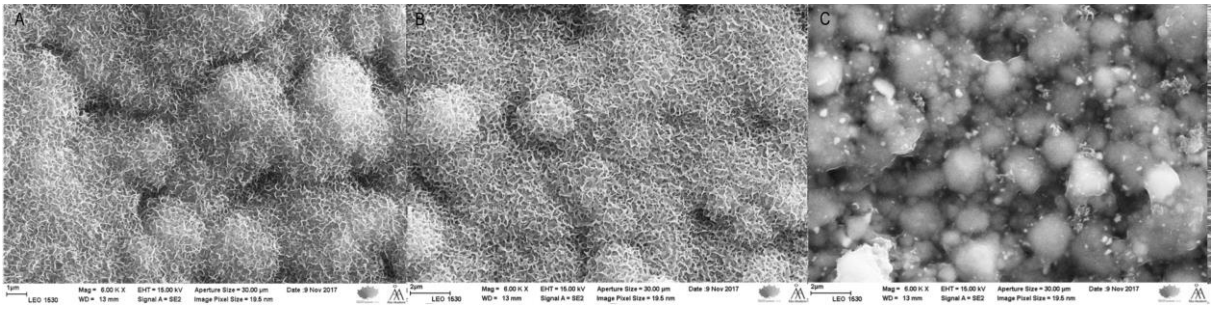


**Fig. 3** Scanning electron microscopy (SEM) images of biomineralized surfaces of A. EDTA treated; B. FCC grit blasted; C. composite coated dentine surface. Original magnification x6000, bar A=1 μm, bar B&C = 2 μm

There was no statistically significant difference between the cut surface with smear layer specimens and the FCC grit blasted samples in area ($p$=0.256) or diameter ($p$=0.256). A statistically significant difference was found between EDTA and FCC specimens, and between EDTA and cut surface with smear layer in both parameters (diameter and area) ($p$<0.001). FCC grit blasted and EDTA treated specimens both showed hydroxyl apatite formation after SBF incubation for 7 days. Composite-coated dentine specimens had no hydroxyl apatite formation (Fig. 3). EDX analysis revealed that the composite-coated dentine specimens did not have any calcium or phosphorus in their composition. Figure 4 shows the analysis made using SEM to quantify tubule occlusion on the samples evaluated.

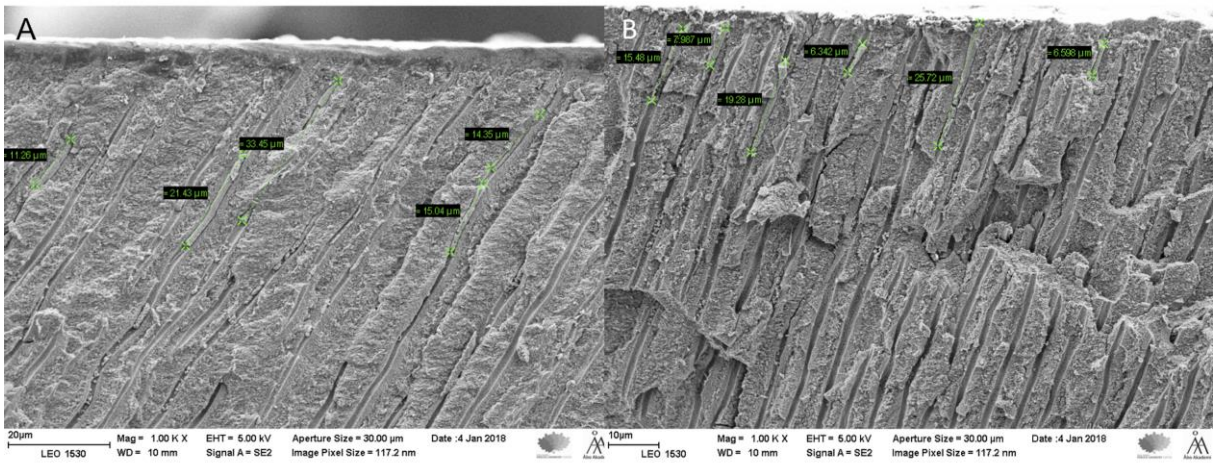


**Fig. 4** Scanning electron microscopy (SEM) images of tubule occlusion analysis of cross section of dentine samples. A. FCC grit blasted specimens; B. EDTA treated after being mineralized in SBF. Original magnification 1.000x, bar=20 µm

## 3.2 X-ray diffraction characterization

XRD characterization demonstrated the blue vertical lines (Figure 5) that are diffraction patterns from a database and corresponds to hydroxyl apatite ($Ca_5(PO_4)_3OH$, JCPDS card 01-076-0694).

# 4. DISCUSSION

The close relationship between dentine hypersensitivity (DH) and dentinal tubule fluid has been documented. The movement of this fluid is associated with the tubular radius and the pressure difference between the two ends of the tubule[33][34]. Accordingly, a technique or material that facilitates the reduction of the radius of dentinal tubules, or that promotes the occlusion of dentinal tubules is considered as capable of reducing dentine permeability. As a result, it is expected to be effective in treating DH. A considerable amount of desensitizing products such as fluoride, oxalate or strontium salts have demonstrated their ability to occlude dentinal tubules *in vitro* and *in vivo*[35][36][37]. However, poor effectiveness and short durability are some of the drawbacks that can be identified in some of the products that are used to treat DH. Saliva naturally occludes dentinal tubules by carrying calcium and phosphate ions into the tubules to promote tubule plugging [38]. However, this natural process of tubule occlusion is slow and the tubule plugging can be affected by dietary acid and physical mechanisms, not providing lasting relief of DH. Novel materials are still needed to overcome these problems and to offer long-lasting effects that counteract DH.

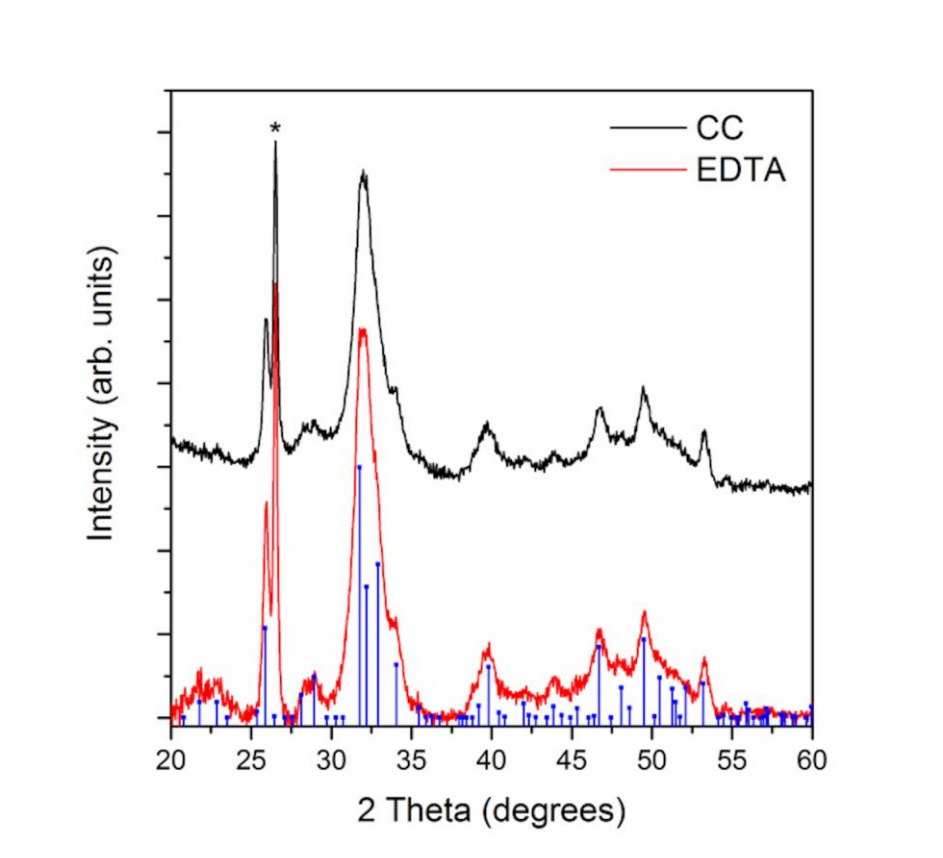

**Fig. 5** XRD diffractograms of functionalized calcium carbonate samples (CC shown in black) and EDTA (red). The asterisk (*) indicates an impurity phase of graphite after carbon sputtering.

This study has investigated the effect of functionalized calcium carbonate (FCC) aggregates on the occlusion of dentinal tubules after tribochemical grit blasting and after biomineralization in simulated body fluid (SBF). The magnitude of occlusion of dentinal tubules within each experimental group was measured using SEM in combination with digital image analysis (Phenom-World, PoroMetric), calculating tubule count, tubule diameter (μm) and area. Tubular occlusion was detected in the FCC grit blasted group with a statistically significant higher ability for occluding the tubules when compared with the EDTA treated samples. The dentine tubule area and diameter were also decreased in the FCC grit blasted group compared with the ones treated with EDTA. This is due to the entrapment of FCC particles to the dentine surface. Most likely, the occlusion is done primarily due breakage of FCC particle of Hap and CC and penetration of the core of the particle. The size of the core of the FCC particle is approx. 1 micrometer that matches to the size of dentine tubule.

The present investigation revealed the presence of precipitates of FCC particles covering the treated dentine surface, occluding the orifices of the dentinal tubules. The FCC particles might have been displaced towards the tubule opening by hydraulic pressure during the grit blasting procedure. The pressure might have forced the particles against each other to create a plug that reduces fluid filtration. It is worth noting that, after fracturing of FCC treated dentine specimens, it was not always possible to find evidence of sub-surface particle inclusions, since their size may have resulted in their loss during specimen preparation. This requires further investigation.

Products that deliver calcium and phosphate may induce intratubular mineralization. Solutions that deliver high concentrations of mineral have shown their capacity to reduce dentine permeability *in vivo*[24]. Hydroxyl apatite formation has been found after the use of materials that interact with biological fluids releasing $Ca^{++}$ ions[39]. In the present study, evidence of biomineralization was identified as hydroxyl apatite was produced in the FCC grit blasted and EDTA treated specimens after SBF incubation for 7 days. Composite-coated

dentine specimens (control group) did not show any evidence of hydroxyl apatite formation during the 7 days incubation period. This is an interesting finding because resin composites that have bisGMA as the main monomer are relatively hydrophilic. In some other applications resin composites of this kind have shown biomineralization to occur in SBF, however, in this study did not contain only bisGMA but also oligomer poly(trans-4-hydroxyl-L-proline) amide which may have caused biomineralization [40]. The present study was not able to demonstrate any microscopically visible difference between the biomineralization of FCC treated versus EDTA treated surface. It is possible that both surfaces provided good enough substrate for biomineralization to occur when the SBF environment last 7 days. It is possible that the dentine substrate with primarily occluded tubules by FCC could have enabled faster biomineralization than the control surface. If this would have been the case, the FCC grit blasting procedure could have a clinical impact in the treatment of DH.

From the biochemical perspective of development of dentine, the creation of a collagen matrix that afterwards mineralizes represents the initial event in the formation of new dentine. After the mineralization of the matrix, the secretion of highly phosphorylated proteins into the matrix by the odontoblasts takes place[41]. When the dentine is biomineralized afterwards like during the treatment of DH, the binding process of calcium follows the same principles:  the phosphate groups bind calcium, which leads to the mineralization of the matrix[42]. A desirable treatment for dentine sensitivity should induce dentine changes that mimic the natural desensitizing effect found in human teeth, where after dentine exposure a spontaneous occlusion of the tubules occurs. Further studies are needed to demonstrate whether the initial occlusion of the dentinal tubules by grit blasting has an effect on the decrease of dentinal permeability. It is also needed to identify if the grit blasted surface can become better biomineralized in less optimal conditions than in an *in vitro* SBF environment of 7 days.

# CONCLUSIONS

The present study demonstrated the effectiveness of FCC-Hap grit blasting in occluding dentinal tubules initially after grit blasting. In a simulated biomineralization environment, the dentine surface was covered with a layer of hydroxyl apatite; however, the 7 days SBF environment no differences were found between coverage by hydroxyl apatite of the FCC-Hap blasted specimens and the control group.

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