Comparison of Dentin Caries Remineralization with Four Bioactive Cements

Keywords

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ABSTRACT

The treatment of deep carious lesions involves the use of ion-releasing agents to seal the lesions. These agents release minerals, leading to the remineralization of the remaining demineralized dentin. This study aimed to compare the dentin caries remineralization with bioactive cements. 60 Dentin blocks were prepared from the dentin of human third molars. Artificial carious lesions were induced on the blocks with pH cycling. The samples were divided into five groups (n=12). Dycal, Oxford ActiveCal PC, Biodentine, and ACTIVA BioACTIVE were applied using a mold. One group did not receive any cement. The samples were stored in remineralization solution for 30 days. The cement was removed using a #15 blade, and the dentin surface was evaluated using Energy-dispersive X-ray Spectroscopy and X-ray Diffraction. One-way ANOVA did not show a significant difference in the weight percentages of calcium and phosphorus and the calcium-to-phosphorus ratios between the groups. The highest and the lowest weight percentages of calcium and phosphorus were observed in Biodentine and control groups, respectively. There were no significant differences in the remineralization properties of bioactive cements. Hydroxyapatite crystals were not formed in any of the adjacent dentin using these cements.

INTRODUCTION

Restoration of deep carious lesions is challenging due to the risk of pulp exposure and endangering its vitality.[1] In the past, treatment of deep caries often resulted in pulp exposure and root canal treatment. Endodontic treatment negatively affects the longevity of restored teeth during oral function.[2] In recent years, with the introduction of the minimal intervention (MI) concept in restorative dentistry to preserve more dentin and the vitality of the pulp, the methods used to treat caries have also changed.[3]

The objective of conservative dentistry is to remove the outer dentin layer (infected dentin), preserve the inner dentin layer (affected dentin), and remineralize it with the help of novel dental materials.[4] The selective caries removal protocol has been proposed as a modern method for deep caries to achieve this goal. In this technique, caries is completely removed from all walls except the walls adjacent to the pulp. To prevent pulp exposure, demineralized dentin is left in the areas close to the pulp and sealed with special restorative materials.[5]

Of the new techniques in conservative dentistry, the use of bioactive materials has been proposed as a means to stimulate the remineralization of affected dentin.[6] Dentin remineralization is more difficult compared to enamel remineralization due to the presence of an organic matrix. Most materials used to remineralize affected dentin include a solution of calcium and phosphate and various concentrations of fluoride ions. Remineralization occurs with the epitaxial growth of residual apatite crystals in partially demineralized dentin, indicating that remineralization does not occur in the absence or presence of small amounts of crystals.[7]

Therefore, it is important to evaluate the ability of new pulp capping materials to remineralize and regenerate dentin. Proper interaction of calcium silicate cements with the remaining affected dentin results in biological remineralization of the carious lesion if the pulp is vital.[8]

Oxford ActiveCal PC is a pulp capping material containing MTA filler, which has been reinforced with a light-cured resin. The manufacturer claims that it has bioactive properties similar to MTA and stimulates the formation of hydroxyapatite. Its thixotropic properties allow an easy and precise application with a needle.[9] This material has recently been introduced, and so far, according to literature reviews, no study has been carried out on its bioactive properties in comparison with other available cements and its effectiveness in dentin remineralization.

Biodentine is another calcium silicate cement that has been introduced to solve common MTA problems. According to the manufacturer, this material has properties similar to dentin and is suitable for replacing carious dentin. This substance has been recommended for use in pulp capping treatment.[10,11]

Activa BioACTIVE is a bioactive material with an ionic resin matrix and bioactive glass fillers. According to the manufacturer, this material can release and recharge calcium, phosphate, and fluoride ions and stimulate remineralization on the tooth surface.[12,13]

A limited number of studies have evaluated the effectiveness of these materials on remineralizing carious dentin lesions in indirect pulp capping with contradictory results with different materials. Fallahzadeh *et al* (2019) reported that Biodentine was not successful in remineralizing carious dentin.[14] In contrast, Kermanshah *et al* (2020) reported that Biodentin increased the weight percentage of calcium and phosphorus of the adjacent carious dentin.[15] Considering the importance of carious dentin lesion remineralization in conservative restorative treatments and various materials that have been introduced by various manufacturers to stimulate dentin formation, the present study aimed to evaluate and compare the remineralization properties of Biodentine, Oxford ActiveCal PC, Dycal, and Activa BioACTIVE cements.

The null hypotheses of the study were as follow:

1. The type of cement does not affect the mineral content of demineralized dentin.
2. The type of cement does not affect the formation of hydroxyapatite crystals in demineralized dentin.

MATERIALS AND METHODS

SAMPLE PREPARATION

Fifteen third molar teeth extracted for reasons other than caries were collected. The teeth were disinfected in an 0.5% chloramine T solution for four weeks at 4°C and then kept in the normal saline solution until the experiments for a maximum of three months. The crowns were removed at the CEJ area with diamond discs in high-speed handpieces under water and air cooling to prepare dentin discs. The crowns were mounted in an acrylic mold. A Mecatome T201 was used to first remove the occlusal third of the teeth and then cut from the circumference so that four dentin discs with 2×2-mm dimensions were obtained from the dentin (1-1.8-mm distance from the pulp chamber roof) of each tooth. The upper surface of the dentin blocks was polished with silicon carbide paper (180, 320, and 600 grit). Due to the small size of the samples, the samples were mounted with wax (Polywax) for easy testing so that only the upper surface remained exposed.

DEMINERALIZATION PROCESS

The samples underwent pH cycling for 14 days. For this purpose, the samples were immersed in a demineralizing solution (containing 2.2 mmol $CaCl_2$, 2.2 mmol NaH_2PO_4, 0.05 mol acetic acid, and 1 mol KOH at pH=4) for 8 hours and then transferred into a remineralizing solution (containing 1.5 mmol $CaCl_2$, 0.9 mmol NaH_2PO_4 and 0.15 mol KCl at pH=7) for 16 hours[16].

REMINERALIZATION AND RESTORATION PROCESS

Materials used in this experiment for remineralization of carious dentin included Dycal, Oxford Activecal PC, Biodentine, and Activa Bioactive; one group did not receive any substance and was considered as control group. The samples were divided into five groups (n=12).

In group D, a mold with a 2×2-mm dimension- and 0.5 mm depth was placed on the demineralized part of the dentin, and Dycal cement was applied on the dentin using this mold. To prepare the cement, an equal amount of base and catalyst paste of Dycal was mixed on a paper pad and applied on the samples with a spatula inside the mold.

In group AC, Oxford Activecal PC cement was applied to the demineralized surface with a similar mold and cured according to the manufacturer's instructions for 40 seconds with a light-curing device (Cordless Curing Light System) at 1000-mW/cm^2 intensity and the 430-490-nm wavelength.

In group B, Biodentine was prepared according to the manufacturer's instructions and applied on the upper surface of the samples using the mold. The cement surface was covered with a moist cotton for 15 minutes to complete the setting.

In group AB, Activa Bioactive was applied to the samples using the mold and cured according to the manufacturer's instructions for 20 seconds with a Cordless Curing Light System at 1000-mW/cm^2 intensity and 430-490-nm wavelength.

Wax was placed on the samples of the control group (group C) with the same mold. The samples were then stored in a remineralizing solution containing 1.5 mmol $CaCl_2$, 0.9 mmol NaH_2PO_4, and 0.15 mol KCl at 37°C for 30 days. The solution was renewed daily.

After 30 days, the cements were removed from the surface of the specimens using a #15 blade. To compare the mineral content of dentin, the surface of all samples was quantitatively analyzed by energy-dispersive x-ray spectroscopy (EDX, Tescan FE-SEM MIRA3). To evaluate the formation of hydroxyapatite crystals during remineralization, x-ray diffraction analysis (XRD, X'Pert PRO MPD) was performed on two samples from each group.

RESULTS

Remineralization of carious lesions in dentin with bioactive cements was evaluated using EDX-SEM and XRD tests. An example of SEM-EDX images taken from each group is shown in Figure 1. The dentin surface in the control group *(Figure 1A)* remains demineralized, and dentin tubules are completely exposed, whereas in the groups treated with bioactive cements, mineral deposition and relative sealing of the dentinal tubules are observed.

In the SEM image of the dentin treated with Dycal *(Figure 1B)*, mineral deposits in the opening of dentin tubules and the surface of demineralized dentin are visible. Fine and diffused sediments are seen on the surface of dentin remineralized with ACTIVA BioACTIVE *(Figure 1C)*, and the second lowest rate of closure of dentinal tubules was seen in this group after the control group. Granular mineral deposits are extensively formed on the dentin surface remineralized with Oxford ActiveCal PC *(Figure 1D)*. These deposits have formed irregular external borders and reduced the diameter of the dentinal tubules. The SEM image of dentin remineralized with Biodentine shows that the dentin surface is covered by mineral particles of various sizes and shapes, leading to the complete occlusion of most dentinal tubules *(Figure 1E)*.

The average weight percentages of calcium and phosphorus obtained from the EDX test for the samples of each group are listed in Table 2. Due to the normal distribution of data, one-way ANOVA test with a significance level of 0.05 was used to compare the mean weight percentages of calcium and phosphorus and the calcium-to-phosphorus ratio in each group. The results showed no significant differences in the weight percentage of calcium (P=0.47) and phosphorus (p=0.52) and the calcium-to-phosphorus ratios (P=0.08) between the groups.

The diagram obtained from the XRD test is shown in Figure 2. The 32º peak of hydroxyapatite was present in all groups, and no difference in peak intensity was observed between the groups. The structure of dentin in the control group was relatively amorphous, indicating the relative demineralization of dentin. The 29º peak seen in the diagrams of dentin adjacent to Biodentine, Dycal, and Oxford ActiveCal is attributed to calcium carbonate. Comparison of the graphs of different groups did not show a significant change on dentin surface after applying different cements, and no evidence of new hydroxyapatite crystals was obtained.

DISCUSSION

Restorative treatment of deep carious lesions is challenging due to the risk of pulp exposure.[1] The selective caries removal protocol has been proposed as a modern method for the treatment of deep caries. In this technique, demineralized dentin is left in the areas close to the pulp to prevent pulp exposure and sealed with special restorative materials to allow remineralization of the remaining carious dentin.[5] In this study, the remineralization capability of demineralized dentin was evaluated and compared by four bioactive cements, including Dycal, Biodentine, Activa BioACTIVE, and Oxford ActiveCal PC. The results showed that although the mean weight percentage of calcium and phosphate ions in the dentin interface was higher in Oxford ActiveCal PC and Biodentine samples, it was not significantly different from the other samples.

In this study, the pH cycling method was used to induce artificial caries in dentin samples. This method creates demineralized dentin with intact collagen, similar to the inner layer of carious dentin. Marquezan *et al* found that the depth of demineralization created in the pH cycling method was higher than using acid gel because the periodic renewal of demineralization and remineralization solutions prevents their saturation by the released ions. The microhardness values in the pH cycling samples were similar to the natural affected carious layer.[16] Keeping the dentin collagen matrix intact is a potential advantage of *in vitro* biomineralization studies.[17,18] Studies have shown that collagen structure plays an active role in the conduction and growth of apatite crystals. Charged groups in the collagen structure stimulate the formation of apatite cores by providing deposition sites. The three-dimensional arrangement of the charged groups in the dense collagen matrix creates an epitaxial pattern that can mediate the crystallographic orientation of the crystals.[18]

The release of calcium ions by restorative materials used in pulp capping treatments plays an essential role in the effects observed following the use of these materials.[19]

Previous studies have shown the formation of mineral crystal structures by cements used in pulp capping treatments in response to the surrounding environment. Granular deposits on the surface of calcium hydroxide have been observed in histochemical studies.[20] Large granules are

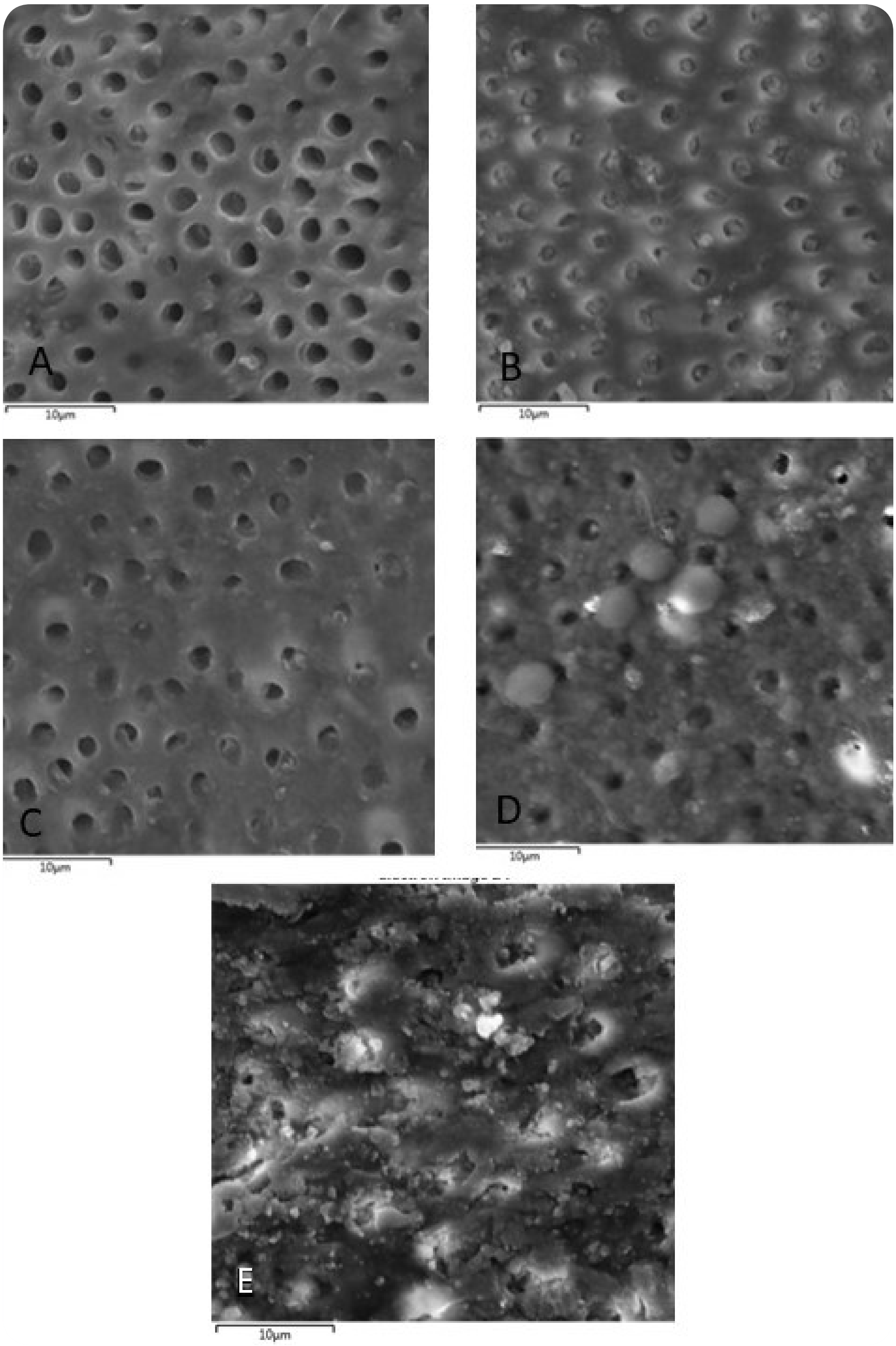

Figure 1: SEM images: Demineralized dentin (A), Dycal-treated dentin (B), ACTIVA BioACTIVE-treated dentin (C), Oxford ActiveCal PC-treated dentin (D), Biodentine-treated dentin (E)

mainly composed of calcium carbonate or calcium phosphate deposits and can enhance the deposition of calcium salts by the pulp and provide more favorable conditions for the differentiation of odontoblasts and dentin deposits.[21] In addition, due to its similarity to biological apatite in bone, cementum and dentin can help initiate dentinogenesis.[20]

Calcium silicate-based cements, are perfect alternatives to calcium hydroxide-based materials, as favorable clinical results have been observed following their application in pulp capping treatments.[22]

The rate of ion release from bioactive materials depends on the nature of the mineral particles and the structural network of the cement that is responsible for water uptake and diffusion, and solubility. Dycal setting reaction leads to the formation of calcium salicylate. The presence of this resin phase causes less solubility of the substance.[23] However, a significant amount of calcium is released from Dycal due to the hydrophilic nature of the substance and its susceptibility to hydrolytic degradation.[24] Therefore, Dycal releases calcium and hydroxyl ions after contact with water, making the pH of

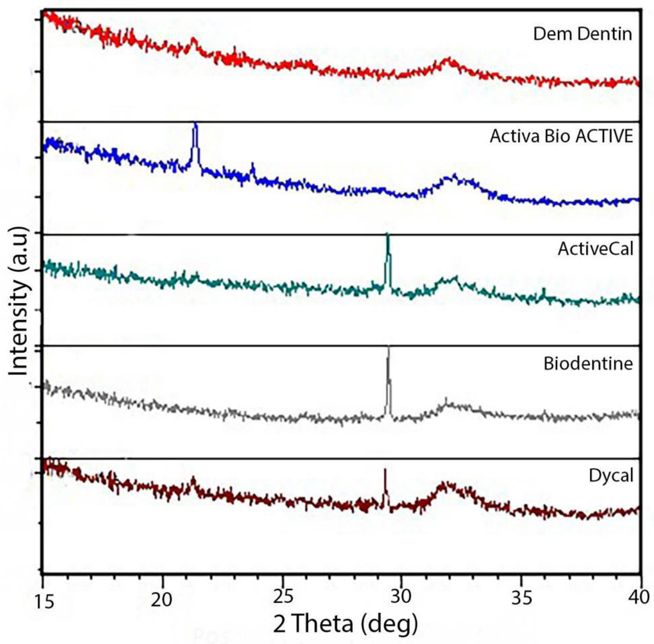

Figure 2: Diagram of the XRD test of the samples.

the environment alkaline, which is associated with the formation of calcium phosphate precipitate by this substance.[25] Gandolfi reported high calcium ion release from Biodentine and alkaline activity. The release of large amounts of calcium was attributed to its presence in the chemical composition of the substance and its rapid hydration reaction. In this study, calcium silicate-based materials released higher amounts of calcium and hydroxyl ions than calcium hydroxide-based materials with a resin component, and the atomic calcium-to-phosphorus ratio in deposits formed on the surface of calcium silicate materials was higher and closer to that in carbonate within bone apatite.[23] The results of the present study also confirmed the greater release of calcium ions, followed by remineralization of dentin by calcium silicate cements (Biodentine and Oxford ActiveCal) compared to Dycal. However, there was no difference in the calcium-to-phosphorus ratio between the groups.

The bioactive property of Activa BioACTIVE is attributed to the release and recharging of significant amounts of calcium, phosphate, and fluoride ions.[26] The present study showed a lower remineralization capacity in Activa BioACTIVE than silicate cements. Studies have shown that the release of phosphate and fluoride ions from Activa BioACTIVE is lower than resin-modified glass-ionomers (RMGI) during the first 24 hours after recharging. The existence of a resin component, lower solubility, formation of hard-to-dissolve calcium fluoride and calcium phosphate surface layer are some of the reasons for this issue.[27] Jun *et al*. compared the biomineralization property of Activa BioACTIVE, Theracal, and Dycal. Alkaline phosphatase activity and biomineralization in the presence of diluted Activa BioACTIVE extract were significantly lower than Dycal. The calcium and hydroxyl ions in Dycal and Theracal samples were significantly higher than in Activa BioACTIVE.[12] Lower ion release indirectly indicates less remineralization capability. Consistent with the present study, ion release and remineralization of Activa BioACTIVE is lower than calcium silicate cements.

In the study of Schwendicke *et al* to compare the remineralizing effect of glass-ionomer, Biodentine, ProRoot MTA, and an experimental resin adhesive containing bioactive glass, no significant difference was observed between the groups in the mineral gain of dentin adjacent to bioactive materials. Examining the samples under a focal microscope showed that none of the materials could completely remineralize the artificial carious lesion. Sedimentation of minerals in the upper surface of dentin treated with Biodentine and MTA was visible in TEM images.[4] In the present study, the weight percentage of calcium and phosphorus in the samples treated with bioactive cements, except for the Activa BioACTIVE group, was higher than the control group. However, there was no significant difference between the materials.

The results of this study are consistent with the study of Li *et al* to some extent. In the Li's study, no significant difference was observed in the Raman diagram of dentin remineralized by Biodentine, ProRoot MTA, and TheraCal LC. However, the rate and intensity of induction of remineralization by resin-free calcium silicate cements (Biodentine and ProRoot MTA) were significantly higher than the cement with a resin component (TheraCal LC). Since in previous studies, the rate of calcium ion release from Biodentine was reported more than TheraCal LC, this difference between resin-based and non-resin-based cements was attributed to the difference in calcium release rate by these cements. In this study, no significant difference in dentin remineralization was observed between 1 week, and 1, 3, and 6 months storage intervals in SBF.[17]

On the other hand, Fathy reported a significant increase in calcium and phosphate ions in demineralized dentin in contact with Biodentine compared to TheraCal after six months. Higher content of calcium silicate in Biodentine compared to TheraCal LC, lack of water in the chemical composition of TheraCal LC and complete dependence on water uptake from the environment, limitation of water release from dentin-pulp complex to the set material, and the limitation of ion release by polymerized resin matrix have been mentioned to justify the results.8 However, in the present study, Oxford ActiveCal, despite having a resin component, induced remineralization similar to Biodentine. This difference in results can be attributed to differences in chemical composition and how the calcium silicate component is incorporated into the resin matrix in ActiveCal Oxford, as well as differences in sample design, how the experiment is performed, and the duration of the remineralization phase.

The graphs obtained from the XRD test did not confirm the formation of hydroxyapatite crystals on the dentin surface treated with the cements. Despite the 32° wide peak in the diagram of dentin samples treated with bioactive cements, it is not possible to confirm the formation of new hydroxyapatite crystals because other peaks related to hydroxyapatite have not been recorded. The absence of crystal structures in the SEM images confirms the results obtained from the XRD test. The main phase formed at the surface of dentin specimens in the Biodentine, Oxford ActiveCal, and Dycal groups is calcite (20–29.5°, $CaCO_3$, PDF #85-1108) which can inhibit the formation of calcium phosphate deposits.28 This finding was not unexpected due to the presence of calcium carbonate in the chemical composition of Biodentine and the formation of this compound during the reaction between the cements and the surrounding environment.

During the setting reaction of calcium silicate cements, a calcium-rich surface is formed on the surface of the cement. Exposure of the set cement to the phosphate-containing solution, HPO_4^{2-} ions are absorbed to the calcium-rich surface of the cement, and the supersaturated solution of calcium and phosphate ions are formed, resulting in the formation of amorphous calcium phosphate. Over time, amorphous calcium phosphate is first converted to octalcium phosphate and then to apatite. The apatite is a calcium-deficient carbonated apatite type with a weak crystalline structure. Various studies have reported a variety of morphologies for the formed calcium phosphate phase. These differences can be due to the presence of amorphous calcium phosphate, octacalcium phosphate, or calcium-deficient apatite with varying degrees of replacement of cations such as Na^+ and Mg^{2+} and anions such as Cl^-. This variation occurs in the calcium phosphate phase, and its weak crystalline structure might explain the relatively amorphous and crystal-free structure of dentin treated with bioactive cements in this study.[29] Kim *et al* reported the presence of amorphous calcium phosphate at dentin-MTA and dentin-Biodentine interface after immersion of root dentin samples whose root canal was filled with MTA and Biodentine in SBF (simulated body fluid) for four weeks. In this study, the calcium-to-phosphate ratio in the interfacial layer of dentin-Biodentine was measured at 2.7 by EDX, which is similar to the ratio in the present study (Ca/P ratio = 2.2).[30]

The calcium-to-phosphorus ratio in all the five groups was calculated at about 2. However, the calcium-to-phosphorus ratio in hydroxyapatite crystals is 1.67 stoichiometrically, and the hydroxyapatite crystals ratio in the human body is 1.5 to 1.7. This difference in the calcium-to-phosphorus ratio shows that hydroxyapatite was not formed in the interfacial layer of cement-dentin in the samples. On the other hand, XRD analysis of deposits formed on the surface of calcium silicate cements in other studies has shown that these deposits are hydroxyapatite.[31] Therefore, there is a possibility that the deposits formed at the material surface are different from those formed at the cement-dentin interface because the phosphate in the environment cannot be in contact with the cement-dentin interface as much as it is in contact with the cement surface.[30] It is also important to note that solutions with different phosphate concentrations in different studies have been used. However, to form hydroxyapatite with a structure and composition similar to that found in the human body, a solution with a concentration of phosphate similar to or close to blood plasma should be used.[32] Therefore, the results of studies that have used phosphate solution with concentrations different from plasma to evaluate the bioactivity of calcium silicate cements should be used with caution.[33]

In this study, the remineralization property of four bioactive cements in carious dentin was investigated. Evaluation of dentin remineralization is a challenging process because there is no standardized protocol for sample designing, how to induce demineralization in dentin, the solution used for remineralization, and the period of remineralization. Also, there are no quantitative and reliable tests to evaluate the degree of remineralization. The differences in the mentioned areas between different studies have led to differences in the results. It seems that more studies are needed to identify the mineral phase formed by bioactive materials on the dentin surface and with a maximum simulation of clinical conditions. The use of samples with living pulp tissue and simulation of pulpal pressure and induction of demineralization by cariogenic microbial plaque may help obtain more accurate and clinically relevant results.

CONCLUSION

Under the limitations of this study, there was no difference in the dentin remineralization properties of the studied bioactive cements. During the 30-day remineralization period, hydroxyapatite crystals were not formed in any of the adjacent dentin of the cements.

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MANUFACTURER'S DETAILS

- Dycal (Densply, USA)
- Oxford ActiveCal PC (Oxford Scientific, Germany)
- Biodentine (Septodont, USA)
- ACTIVA BioACTIVE (Pulpdent, USA)
- Diamond discs (Jota, Switzerland)
- Mecatome T201 (A, Presi, Grenoble, France)
- Polywax (Bilkim Co., Ltd., Turkey)
- Cordless Curing Light System (TPC, USA)
- FESEM (Tescan FE-SEM MIRA3, TESCAN, Czech Rep.)
- X'Pert PRO MPD (Malvern Panalytical)

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