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Impact of Bioactive Cements on Mineral Density of Artificially Demineralized Dentin

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ABSTRACT

Objectives: Bioactive dental materials have the potential to remineralize carious dentin. This study compared the ability of four bioactive cements to increase the mineral content of demineralized dentin. Methods: Four cavities (2×0.5 mm) were prepared within the dentin of the occlusal surface of 15 teeth. The samples underwent pH-cycling for 14 days to induce demineralization. Three teeth were randomly assigned to each group (Biodentine, ACTIVA BioACTIVE, Oxford ActiveCal, Dycal, control). After placing the materials in the cavities, the entire occlusal surfaces were covered with composite resin, the samples were stored in a remineralizing solution for 30 days. The mineral density of the pulpal floors was determined using micro-CT. The data was analyzed with one-way ANOVA and Tukey tests. After horizontal sectioning, SEM-EDS mapping was done at the cement-dentin interface of the cavity floor of one sample in each group. Results: There were significant differences between the test groups compared to the control group in terms of the mineral density of the cavity pulpal floors. The highest calcium and phosphorus weight percentages were observed in the Biodentine and ActiveCal groups, respectively. Conclusions: All the bioactive cements increased the mineral content of artificially demineralized dentin at the pulpal floor of the cavities.

INTRODUCTION

Vital pulp therapy (VPT) preserves pulpal tissue that is at risk from caries, trauma, restorative procedures, or iatrogenic reasons and increases tooth survival because it conserves the integrity of the hard dental tissues.[1]

Various medicaments and restorative materials have been used during vital pulp treatments. Calcium hydroxide was one of the first materials to be used because of its antibacterial properties and its ability to prevent future bacterial penetration and injury to the pulp.[2] However, the material has several disadvantages such as inflammation and surface necrosis of the pulp, tunnel defects in the tertiary dentin that lead to an incomplete barrier against recurrent infection, high solubility in oral fluids, and a lack of adhesion to dentin.[3]

More recently, bioactive materials have been used to induce remineralization of carious dentin.[4] The term 'bioactive' in restorative dentistry refers to materials that form a surface layer similar to apatite in the presence of a mineral phosphate solution.[5] The bioactive materials used in restoring demineralized dentin should provide mineral ions, bind to collagen (to function as a pattern for calcium and phosphorous and induce the apatite

crystallization nucleus), protect collagen against destruction, and provide an appropriate pH to support the formation of new mineral deposits.[6] Calcium silicate cements (such as MTA and Biodentine) and Activa Bioactive are among the materials that have been introduced as bioactive for various applications, including indirect pulp capping (IPC).[7–9] Biodentine (Septodont, USA) is a calcium silicate cement introduced to address the common issues of MTA (long setting time, difficult handling, potential color change). According to the manufacturer, this material has dentin-like properties and is suitable as a substitute for lost dentin.[9] Oxford Activecal PC (Oxford Scientific, Germany) is a pulp-capping material containing MTA filler and reinforced with a light-curable resin, which, according to the manufacturer, has bioactive properties similar to MTA and stimulates hydroxyapatite formation. Its thixotropic property allows easy and precise placement, and being light-curable provides controlled setting.[10] Activa Bioactive (Pulpdent, USA) contains a resin-based universal matrix and bioactive glass fillers. According to the manufacturer, this material can release and recharge calcium, phosphate, and fluoride ions and stimulate remineralization at the tooth surface.[8,11]

The remineralization of deep carious dentin in conservative restorative procedures is important, and a range of dentinogenic agents have been introduced by various manufacturers. However, limited studies are available on the efficacy of these materials in increasing the mineral content of deep carious dentin during indirect pulp capping, with unfortunate discrepancies in the results. Therefore, the present laboratory study was undertaken to evaluate and compare the efficacy of Dycal (Dentsply, USA), Oxford ActiveCal PC (Oxford Scientific, Germany), Biodentine (Septodont, USA), and ACTIVA BioACTIVE (Pulpdent, USA) to increase the mineral content on the dentin (pulpal) floor of artificially demineralized cavities in extracted teeth. The null hypothesis is that Dycal, Oxford ActiveCal PC, Biodentine, and ACTIVA BioACTIVE have the same potential to increase the mineral content of artificially demineralized dentin.

METHODS

ETHICAL CONSIDERATIONS

The research proposal was approved by the research ethics committee of the Tehran University of Medical Sciences (IR.TUMS.DENTISTRY.REC.1399.126).

SAMPLE SIZE

According to the results of a previous study 4, using the one-way ANOVA option of PASS 15 software with $\alpha = 0.05$ and $\beta = 0.2$, and considering the effect size to be 0.59, the required sample size for each of the four groups was calculated to be 12. Based on the power calculation, a total of 60 cavities were needed. To achieve this, 4 cavities were prepared on each tooth, resulting in a total of 15 teeth with 4 cavities on each tooth, amounting to 60 cavities in total.

SAMPLES PREPARATION

Fifteen sound-impacted third molar teeth were extracted and included. The teeth were examined under magnification (×20) to ensure they had no enamel cracks, caries, occlusal, or cervical defects. The teeth were stored in 0.5% chloramine T solution at 4ºC until use.[12] First, the roots were removed at the CEJ using a diamond disk (Jota, Switzerland) in a high-speed handpiece under air and water spray. Then, a periodontal probe was used to mark the appropriate location for removing the occlusal surface enamel with a diamond disk in a high-speed handpiece under air and water coolant on each sample to leave a dentin thickness of 2 mm. Four cavities, measuring 2 mm in diameter and 0.5±0.25 in depth, were prepared on the occlusal dentin surface using a 008-diamond bur (Jota, Switzerland) *(Figure 1A)*. The cavity dimensions were controlled using a periodontal probe. All the tooth surfaces including the cavity walls were covered with acid-resistant varnish (Maybelline, New York, NY, USA) except the floors of the prepared cavities.

THE DEMINERALIZATION PROCESS

The samples underwent a pH-cycling procedure for 14 days. Each sample was immersed in 10 ml of a demineralizing solution (consisting of 2.2-mmol $CaCl_2$, 2.2-mmol NaH_2PO_4, 0.05-mmol acetic acid, and 1-mol KOH, pH=4.4) for 8 hours, followed by immersion in 10 ml of a remineralizing solution (consisting of 1.5-mol $CaCl_2$, 0.9-mmol NaH_2PO_4, and 0.15-mol KCl, pH=7) for 16 hours.[13] The solutions were renewed for each cycle.

PLACEMENT OF MATERIALS

Table 1 shows the chemical composition of the materials. Twelve cavities were randomly assigned to each cement (Dycal (Dentsply, USA); Oxford ActiveCal cement (Oxford Scientific, Germany); Biodentine (Septodont, USA); ACTIVA BioACTIVE cement (Pulpdent, USA); Wax (Polywax, Bilkim Co., Ltd., Turkey)). The cavities of each group were marked with a specific color on the external surface of the teeth. The demineralized floor of each cavity was covered with cement to a thickness of 0.5 mm *(Figure 1B)*. The manufacturer's instructions for applying each cement were carefully followed.

Group 1 Dycal (Dentsply, USA): The Dycal was placed in the cavities and left to set.

Group 2 Oxford ActiveCal cement (Oxford Scientific, Germany): The material was placed into the cavities and light-cured for 40 s using a light-curing unit (Cordless Curing Light System, TPC, USA) at a light intensity of 1000 mW/cm2 and a wavelength of 430-490 nm. The light intensity was controlled with a radiometer (Optilux100; Kerr SDS) periodically.

Group 3 Biodentine (Septodont, USA): Biodentine was prepared by adding 5 drops of liquid to the powder and then triturating for 30 seconds, placing it in the 12 cavities, and condensing gently. The cement surface was covered with a wet cotton pellet for 12 minutes to allow the complete setting of the material.

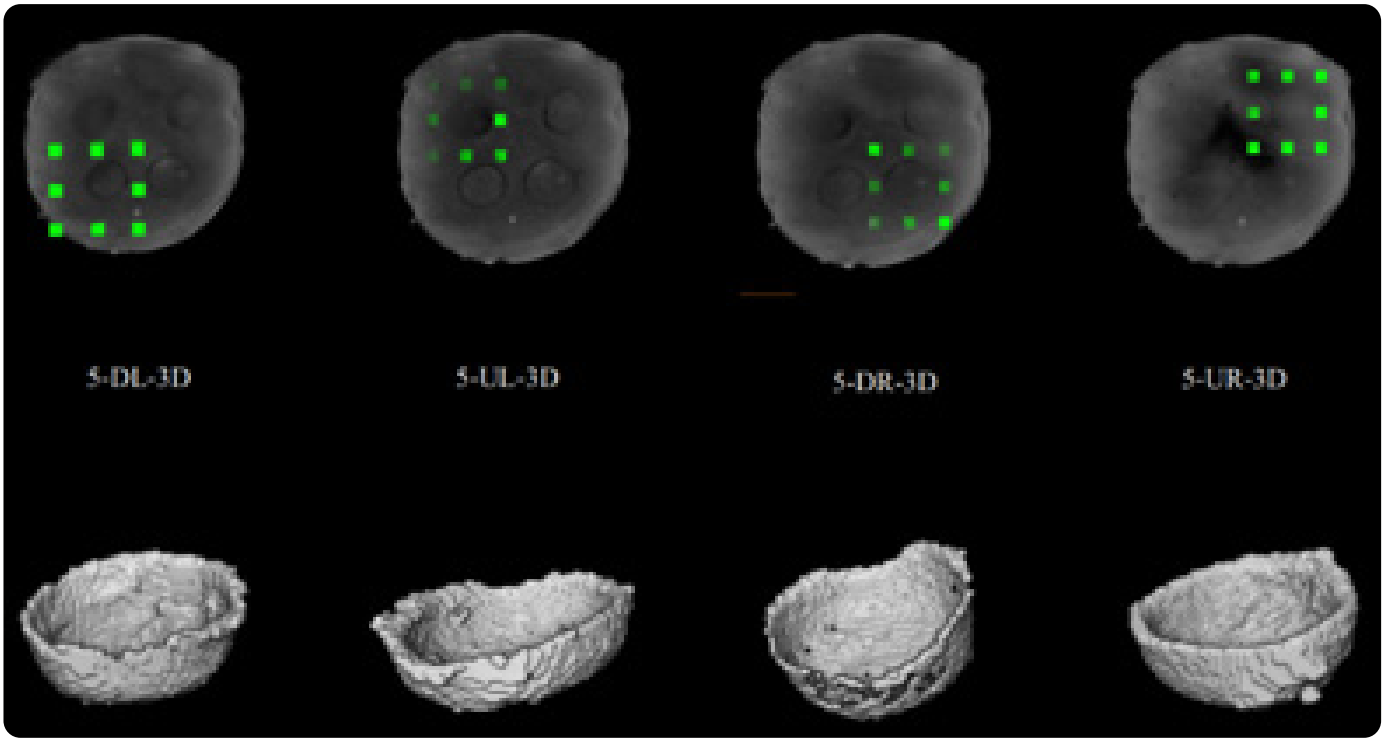

Figure 1: The 3D scan and reconstructed image of one of the study samples.

Table 1. Chemical composition of the materials

	Trade name	Manufacturer	Chemical composition
1	ACTIVA BioACTIVE	Pulpdent, USA	Diurethane dimethacrylate; Bis (2-(Methacryloyloxy) Ethyl) Phosphate; Barium glass; Ionomer glass; Polyacrylic acid/maleic acid copolymer; Dual-cure chemistry; Sodium fluoride; Colorants
2	Oxford ActiveCal PC	Oxford Scientific, Germany	light-cure resin-reinforced MTA pulp capping material
3	Biodentine	Septodont, USA	Powder: tricalcium silicate; dicalcium silicate; calcium carbonate and oxide; zirconium oxide; iron oxide Liquid: water; calcium chloride; hydrosoluble polymer
4	Dycal	Dentsply, USA	Base: disalicylate ester of 1,3, butylene glycol; calcium phosphate; calcium tungstate; zinc oxide; iron oxide Catalyst: calcium hydroxide; ethyl toluenesulfonamide; zinc sterate; titanium dioxide; zinc oxide; iron oxide

Group 4 ACTIVA BioACTIVE cement (Pulpdent, USA): The material in each cavity was light-cured for 20 seconds using a light-curing unit (Cordless Curing Light System, TPC, USA) at a light intensity of 1000 mW/cm^2 and a wavelength of 430-490 nm.

Group 5 Controls: The remaining 12 cavities were filled with wax (Polywax, Bilkim Co., Ltd., Turkey) to act as a control group.

To simulate clinical conditions, the occlusal surfaces of the teeth were etched with 37% phosphoric acid, and then Oxford Bond TE Mono bonding agent (Oxford Scientific, Germany) was applied and light-cured (Cordless Curing Light System, TPC, USA) for 15 seconds. Finally, they were covered with Oxford Ceram Nano composite resin A1 (Oxford Scientific, Germany), and light-cured for 20 seconds (*Figure 1C*). The samples were immersed in a phosphate-buffered solution (consisting of 1.5-mmol $CaCl_2$, 09-mmol NaH2PO4, and 0.15-mol KCl, pH=7) for 30 days at 37°C.[5] The solution was renewed daily.

MICRO-CT IMAGING

After 30 days, a micro-CT scanner (LOTUS-*inVivo*, Behin Negareh Co., Tehran, Iran) was used to determine the radiographic density of the remineralized dentin of the samples. To achieve the highest-quality images, the machine voltage was adjusted to 80 kV, and its current was adjusted to 100 µA. The exposure time was set to 2 seconds, and a magnification of ×2.7 was selected. The samples were fixed so that the longitudinal axis of the teeth was set parallel to the horizontal axis during the scanning. The layer thickness for image reconstruction was adjusted

at 30 µm. All the protocol setting process was controlled using LOTUS-inVivo-ACQ software (Behin Negareh Co., Tehran, Iran). The 3D data were reconstructed using LOTOS in vivo-REC software (Behin Negareh Co., Ltd., Tehran, Iran) with the standard algorithm of Feldkamp, Danis, Knet (FDK). The images were analyzed to determine the mineral density of the dentin adjacent to each cement.

EDS-MAPPING

After the micro-CT scans were obtained, each tooth underwent a transverse sectioning procedure with a Mecatome T201 A (Presi, Grenoble, France) to expose the cement-dentin interface. Any remaining material was removed using a No.12 blade. Then the material-dentin interface on the pulpal floor of each specimen was evaluated with Energy-Dispersive X-ray Spectroscopy (EDX mapping, FESEM, Tescan FE-SEM MIRA3) to find out the distribution and weight percentage of calcium and phosphorus ions. For this purpose, the samples were coated with carbon and examine with SEM (FESEM, Tescan FE-SEM MIRA3).

STATISTICAL ANALYSIS

Dentin radiographic density values of the cavity floors obtained from micro-CT were analysed using IBM SPSS Statistics (Version 25). Data were normally disturbed according to one-sample Kolmogorov-Smirnov analysis. One-way ANOVA and Tukey HSD were performed at a significance level of $P<0.05$.

RESULTS

Figure 2 presents a sample of the reconstructed images after micro-CT scanning of the teeth.

Table 2 presents the mean radiographic densities of the cavity floors. One-way ANOVA revealed a significant difference between at least two groups of samples. Two-by-two comparisons of the groups using Tukey HSD tests revealed significant differences between all four test groups and the control group. However, there were no significant differences between the test groups in terms of the radiographic density of the cavity floors.

Figure 3 presents images obtained from the EDS mapping of the samples. An increase in the amount of ions can be seen on the entire cavity floors in the experimental groups compared to the control group. Uniform distribution of calcium and phosphorus ions on the dentin surfaces suggests that the increase in the weight percentage of the ions is not solely due to the deposition of ions in the dentin tubules. Table 3 presents the wt% of calcium and phosphorus in each group. The highest wt% of calcium was recorded in the Biodentine group (24.32 wt%), and the lowest was recorded in the control group (6.81 wt%). The highest and lowest wt% of phosphorus were recorded in the Oxford ActiveCal (6.21 wt%) and control groups (3.29 wt%), respectively.

DISCUSSION

The present study investigated the change in mineral content of deep demineralized cavities prepared in dentin associated with several bioactive cements using a micro-CT technique. The analysis of the scans of the samples revealed significant increases in the radiographic density of the cavity floors in all the test groups compared to the control group. Bioactive restorative materials can form an apatite-like layer at the material-tissue interface after being immersed in a liquid similar to human blood plasma.[14] Biodentine is an important member of the calcium silicate family of materials, which can be used to replace lost dentin.[9] Studies have reported an increase in the density of dentin mineral content after applying this material.[4,6,15]

Based on studies on TheraCal LC, a light-cured calcium silicate cement, incorporating a resin matrix into a calcium silicate-based cement changes the material's setting mechanism and calcium ion kinetics. A lack of calcium hydroxide formation decreases the material's ability to release calcium ions.[16] Oxford ActiveCal PC is a light-cured resin liner into which MTA has been incorporated as a filler. In the present study, this material successfully increased dentin mineral density, similar to Biodentine, The wt% of calcium and phosphorus in the Biodentine and Oxford ActiveCal samples was similar. Therefore, it appears that the incorporation of a resin component in the Oxford ActiveCal formulation, in contrast to TheraCal LC, had no adverse effect on the release of biologically active ions.

ACTIVA BioACTIVE has been marketed as calcium silicate-based cement with a light-cured resin with a combination of composite resin and glass-ionomer properties. This material activity releases significant amounts of calcium, phosphate, and fluoride in response to pH changes and can be recharged.[17] These mineral ions are responsible for inducing the formation of mineralized hard tissues.[16] Some researchers have called the ability to release biologically active ions biointeractivity, considering it a prerequisite for the bioactivity of a material.[18] According to previous studies, the amount of calcium ions released from ACTIVA BioACTIVE is similar to that of MTA, Biodentine, and TheraCal LC. Therefore, the biomineralization induction potential of this material is similar to other calcium silicate cements.[8] Analysis of the micro-CT results also confirmed similar mineral density in the dentin adjacent to ACTIVA BioACTIVE, Biodentine, and Oxford ActiveCal PC cements. However, the wt% of calcium in Biodentine and Oxford ActiveCal PC was higher than that of ACTIVA BioACTIVE. Nonetheless, it appears this amount has been unable to increase the radiographic density of the samples significantly.

It has been reported that there is no significant difference in the mean mineral density of demineralized dentin treated with GI and Biodentine. 6 However, Neves *et al* reported significantly higher mineral density in demineralized dentin treated with GI than that treated with Biodentine, concluding that the bioactive

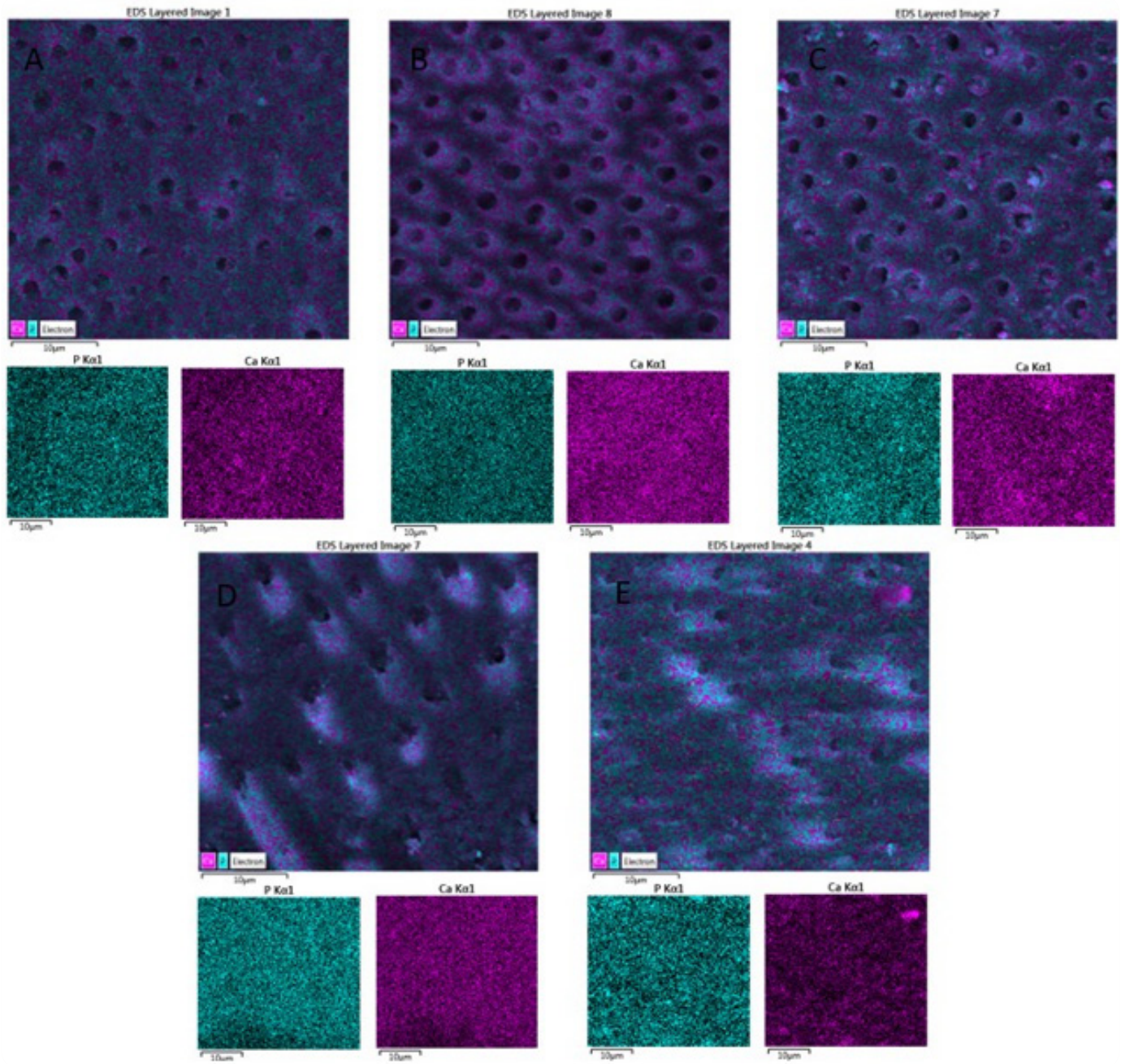

Figure 2: EDS mapping taken from the dentine cavity floor of one sample in each group with × 5000 magnification; A. ACTIVA BioACTIVE; B. Biodentine; C. Dycal; D. Oxford ActiveCal; E. Control. The red color represents Calcium ions, and the green color represents Phosphate ions.

Table 2. Descriptive table of the radiographic densities of the cavity floors of the samples (g/cm^{-3}).

Cements	N	Mean	Min	Max	P value
ActiveCal	12	1.75 ± 0.06 [a*]	1.66	1.83	<0.001
ACTIVA BioACTIVE	12	1.79 ± 0.5 [b]	1.69	1.86	<0.001
Biodentine	12	1.71± 0.11 [c]	1.56	1.93	0.001
Dycal	12	1.70 ± 0.07 [d]	1.62	1.84	0.012
Wax (Control)	12	1.46 ± 0.10 [a,b,c,d]	1.29	1.70	

*Similar superscript letters show significant differences.

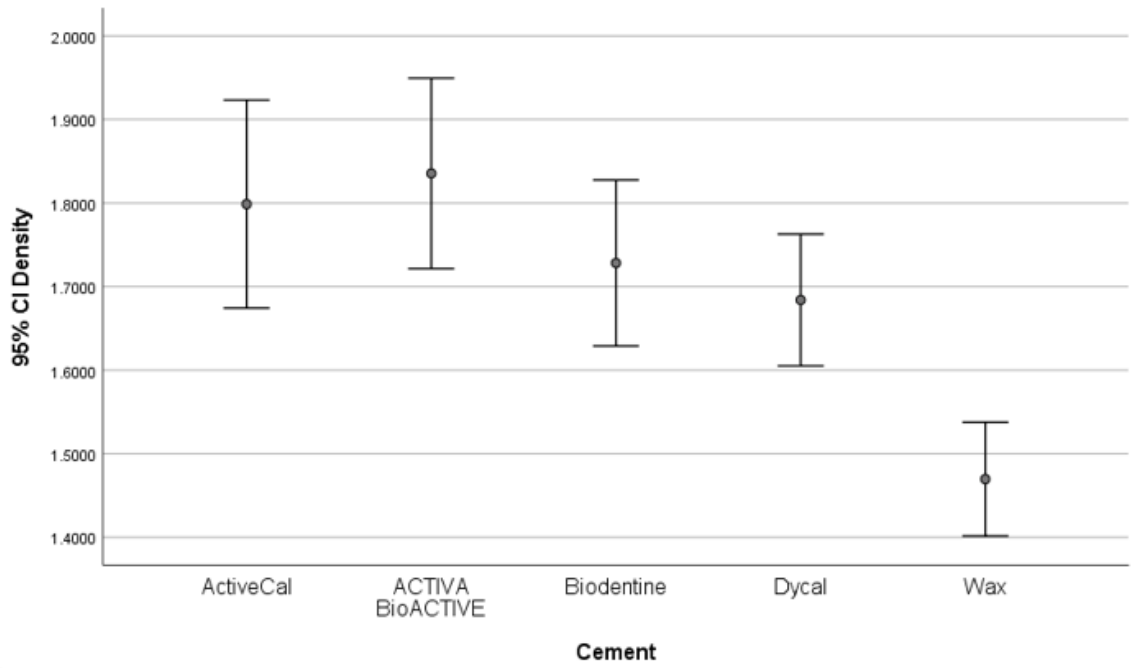

Figure 3: Graph of the mean and 95% confidence intervals of the radiographic densities of the dentin cavity floors beneath the cements.

Table 3. The calcium and phosphate wt% at the cement-dentin interface in the cavity floors.

Cements	Ca (wt%)	P (wt%)
ACTIVA BioACTIVE	12.18	5.88
Biodentine	24.32	5.93
Dycal	11.56	5.75
Oxford ActiveCal	22.51	6.21
Control (wax)	6.81	3.29

potential of Biodentine was less than that of GI. 4 The difference between the results of the two studies might be attributed to differences in study design and analysis. Despite the similarities in the chemical composition of ACTIVA BioACTIVE and RMGI cements, the results of studies on the release of active ions from ACTIVA BioACTIVE compared to GI and RMGI are contradictory.[19,20] Therefore, comparing the biointeractivity and bioactivity between these materials with the available data is not easy. However, the present study did not reveal significant differences in the mineral density of demineralized dentin treated with ACTIVA BioACTIVE and Biodentine and their bioactivity.

SEM-EDS mapping revealed an increase in the amount of calcium and phosphate ions on the entire dentin surface of the experimental groups. Since the distribution of calcium and phosphate ions on the dentin surfaces is uniform, the increase in the weight percentage of the ions is not solely due to the deposition of ions in the dentin tubules. The control group's minimal remineralization highlights that the observed effects were material-driven rather than a passive outcome of the storage solution. Biodentine and ActiveCal exhibited the highest Ca/P wt%, respectively, suggesting their superior ion-releasing capacity. It may be attributed to the tricalcium silicate-based composition of Biodentine and MTA filler in ActiveCal, which forms calcium hydroxide and a hydroxyapatite-like phase upon hydration.[21] The remineralization mechanism by calcium silicate-based cements relies on the release of calcium hydroxide and an alkaline pH. The alkaline pH produced by these cements leads to a caustic effect and increases the permeability of the dentin collagen network, resulting in the penetration of mineral ions and remineralization.[22]

ACTIVA BioACTIVE showed intermediate results, possibly due to its dual mechanism of fluoride release and ionic exchange.[23] Another reason may be the acidic pH of Polyacrylic acid and maleic acid, that has demineralizing effect on dentin, leading to increased dentin permeability and ion penetration.[22] Dycal, a conventional calcium hydroxide liner, had lower remineralization than the other test groups, consistent with its limited long-term bioactive potential.[5]

It has been previously reported that XRD analysis revealed the hydroxyapatite formation at the cement-dentin interface of the samples treated with the same cements.[24] These findings strongly suggest that the cements evaluated successfully increased the mineral content of demineralized dentin.

Higher Ca/P wt% in the adjacent dentin (e.g., in Biodentine and ActiveCal) indicates greater ion release and mineral deposition, which may enhance apatite formation. Lower Ca/P wt% (e.g., Dycal) suggests slower or less effective remineralization, which may not fully stabilize deep caries-affected dentin.[25,26] Longer evaluation periods may reveal differences in mineral density and the remineralization potential of the materials.

According to the present study, the bioactivity of Dycal, Biodentine, Oxford ActiveCal PC, and ACTIVA BioACTIVE cements was the same. Further quantitative and qualitative studies are necessary to compare the quality of demineralized dentin after using these materials and to determine the importance of using bioactive materials to treat deep dentin caries after selective caries removal and indirect pulp capping.

CONCLUSION

Within the limitations of this study, all the studied cements, including Biodentine, Oxford ActiveCal PC, and ACTIVA BioACTIVE exhibited similar capacities to increase the mineral density of demineralized dentin in cavities prepared in extracted impacted third molar teeth.

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