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ORIGINAL ARTICLE

Microstructure and chemical analysis of four calcium silicate-based cements in different environmental conditions

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Abstract

Objective The objective of this study was to analyze the microstructure and crystalline structures of ProRoot MTA, Biodentine, CEM Cement, and Retro MTA when exposed to phosphate-buffered saline, butyric acid, and blood.

Methods and materials Mixed samples of ProRoot MTA, Biodentine, CEM Cement, and Retro MTA were exposed to either phosphate-buffered saline, butyric acid, or blood. Scanning electron microscope (SEM) and energy-dispersive X-ray spectroscopic (EDX) evaluations were conducted of specimens. X-ray diffraction (XRD) analysis was also performed for both hydrated and powder forms of evaluated calcium silicate cements.

Results The peak of tricalcium silicate and dicalcium silicate detected in all hydrated cements was smaller than that seen in their unhydrated powders. The peak of calcium hydroxide ($Ca(OH)_2$) in blood- and acid-exposed ProRoot MTA, CEM Cement, and Retro MTA specimens were smaller than that of specimens exposed to PBS. The peak of $Ca(OH)_2$ seen in Biodentine™ specimens exposed to blood was similar to that of PBS-exposed specimens. On the other hand, those exposed to acid exhibited smaller peaks of $Ca(OH)_2$.

Conclusion Exposure to blood or acidic pH decreased $Ca(OH)_2$ crystalline formation in ProRoot MTA, CEM Cement and Retro MTA. However, a decrease in $Ca(OH)_2$ was only seen when Biodentine™ exposed to acid.

Clinical relevance The formation of $Ca(OH)_2$ which influences the biological properties of calcium silicate cements was impaired by blood and acid exposures in ProRoot MTA, CEM Cement, and Retro MTA; however, in the case of Biodentine, only exposure to acid had this detrimental effect.

Keywords Biodentine · Calcium silicate cement · CEM cement · EDX · MTA · SEM · XRD

Introduction

Calcium silicate cements (CSCs) have various indications of use in endodontics and their potential clinical applications have increased over the years. Mineral trioxide aggregate (MTA) was the first CSC introduced in endodontics. Although having a variety of favorable properties, this CSC has several disadvantages such as difficult handling properties and induction of tooth discoloration [1–4]. Recently, a variety of CSCs have been introduced to overcome these disadvantages.

Biodentine was introduced as a fast setting dentine replacement material [5] with high compressive strength [6, 7]. It is composed of tricalcium silicate, zirconium oxide, and calcium carbonate, which when mixed with its liquid containing calcium chloride and a water-soluble polymer, sets rapidly [8]

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and forms calcium hydroxide ($Ca(OH)_2$) as a by-product of hydration [9].

Calcium-enriched mixture (CEM) Cement was introduced as a white CSC claiming to have no discoloration potential [10–12]. It is composed of CaO (51.75%), SO_3 (9.53%), P_2O_5 (8.49%), SiO_2 (6.32%), and minor components of Al_2O_3, Na_2O, MgO, and Cl [13].

Retro MTA is another fast setting CSC and is said to have less discoloration potential [14]. It consists of calcium carbonate, silicon dioxide, aluminum oxide, and calcium zirconia [15].

The clinical applications of MTA have been suggested for all aforementioned CSCs [5, 16, 17]. In most clinical applications related to the field of endodontics such as root end filling, perforation sealing, vital pulp therapy, and regeneration, CSCs encounter blood and/or in infectious conditions, acidic pH. This contact occurs when these cements are undergoing hydration and maturation [1]. Therefore, knowledge of the effect of these environmental conditions on the chemical compounds and hydration process of these cements is of clinical significance. Some studies have shown that exposure to these conditions affect some properties in different cements [18–20] such as their setting, expansion and physical properties [21]. But data on the effect of different conditions on the properties of Retro MTA and CEM Cement are limited.

The aim of this study was to evaluate the effect of exposure to butyric acid, a metabolism by-products of anaerobic bacteria dominant in endodontic infections and the most common fatty acid found in infectious conditions with endodontic origin [22], and blood during setting on the chemical compounds and hydration process of ProRoot MTA, Biodentine, CEM Cement, and Retro MTA. The null hypothesis was that exposure to blood and acid will not affect these CSCs.

Methods and materials

Plexiglass molds with an internal diameter of 4 and 6 mm heights were fabricated by CNC laser cutting.

Four CSCs were evaluated (Table 1):

1. ProRoot MTA (Dentsply Sirona, Ballaigues, Switzerland)
2. Biodentine (Septodont, Saint Maur des Fosses, France)
3. CEM Cement (Bionique, Tehran, Iran)
4. Retro MTA (BioMTA, Seoul, Republic of Korea)

Three exposure conditions were evaluated:

a. Phosphate-buffered saline (PBS) (pH = 7.4) (Merck, Darmstadt, Germany)
b. Whole human blood (WHB)
c. Butyric acid (BA)(pH = 5.4) (Merck, Darmstadt, Germany)

For preparation of ProRoot MTA and CEM Cement slurries, 1 g of their powder was placed in an empty, clean plastic capsule, and 0.33 mL of the respective liquid was added [23]. For Retro MTA, the powder content of one pack was placed in the same type of plastic capsule and three drops of its liquid were added as suggested by the manufacturer. Biodentine powder is delivered in a capsule, and its liquid is supplied in a single-dose container. Each capsule was mixed with five drops of its liquid. The encapsulated materials were then mechanically mixed for 30 s at 4500 rpm [18] using an amalgamator (Silamat; IvoclarVivadent AG, Liechtenstein).

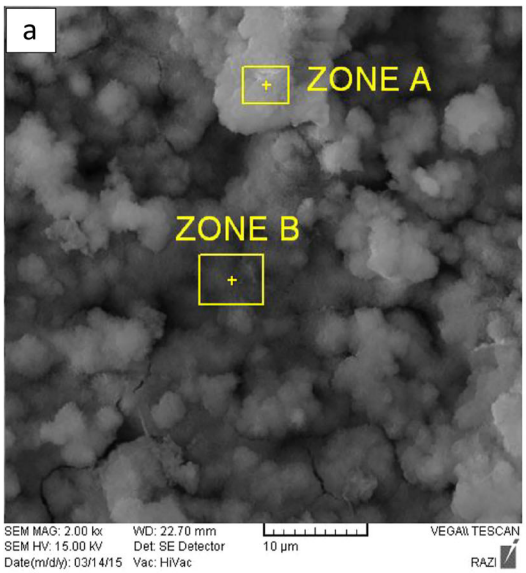
Prior to slurry placement, each mold was filled with either PBS, WHB or BA according to the specific subgroup and excess liquid was aspirated after 20 s. The molds were then filled with the prepared cements using minimal pressure. The materials were then subjected to ultrasonic energy for 30 s at a power scale of 5 using a BUC-1 Spartan tip (Obtura Spartan, Fenton, MO, USA) attached to a Suprasson_P5 Booster (Satelec, Merignac, France) [18, 23]. After initial setting, specimens were placed in Eppendorf tubes containing the respective exposure liquid: PBS, WHB, or BA. Placement was in a way that the lower surface of each specimen would be in contact with the liquid. A moist cotton pellet was then placed over the specimens. They were incubated for 7 days in 37 °C and fully saturated conditions.

After the incubation period, the surface in contact with either PBS, WHB, or BA was coated with gold and analyzed with a scanning electron microscope (SEM) equipped with EDX (SEM-EDX 515, Phillips, Eindhoven, the Netherlands). Images with × 400 and × 2000 magnifications were considered adequate for the characterization of the microstructure. Element analysis was performed on selected segments with crystalline and amorphous structure.

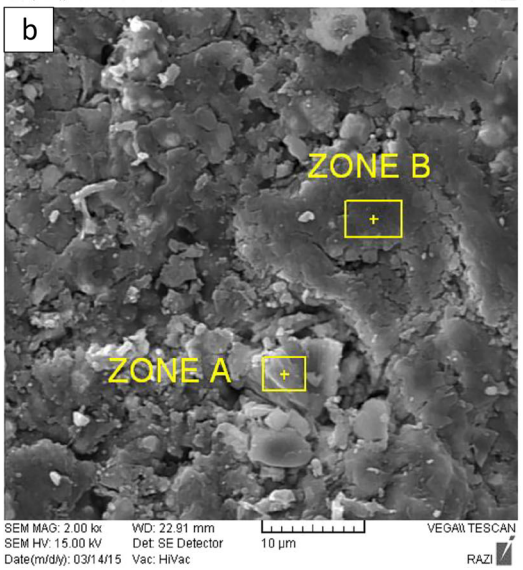
Both hydrated (mixed) and unhydrated (powder) cements were mounted for XRD analysis. The hydrated cements were milled into a fine powder. An automated X-ray diffractometer (X'Pert PRO MPD, Philips) with Cu Kα radiation was set at 40 kVp and 30 mA. Data were collected from scan range of 15–55 with a step size of 0.02°. X'Pert High score plus software (Spectrum Viewer Basic 2.6.3, PANalytical, Nottingham, UK) was used for the analysis. The peaks of each sample were matched with those of the International Center for Diffraction Data (ICDD) database (International Center for Diffraction Data, Newton Square, PA, USA). By comparing the size of each peak representing a crystalline phase between groups, their relative amounts were estimated.

Results

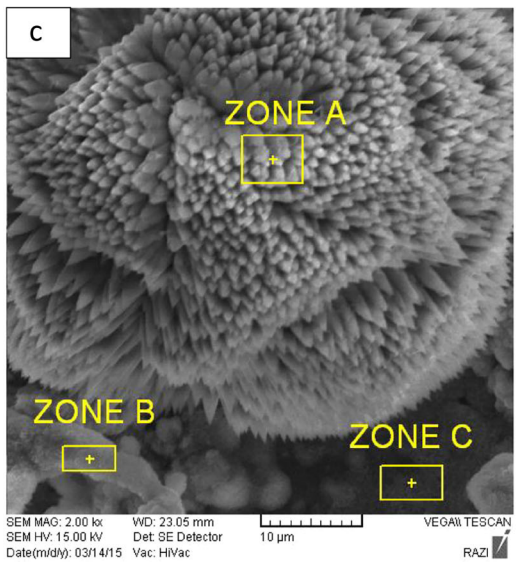
The results of SEM and EDX evaluations of the CSCs exposed to the various media are shown in Figs. 1, 2, 3, and 4.



Element	Wt% in different zones	
	A	B
Carbon	9.38	7.24
Oxygen	45.17	38.03
Calcium	31.53	36.84
Bismuth	4.61	8.23
Silicon	8.78	9.58
Aluminum	0.51	-
Trace elements	0.02	0.08

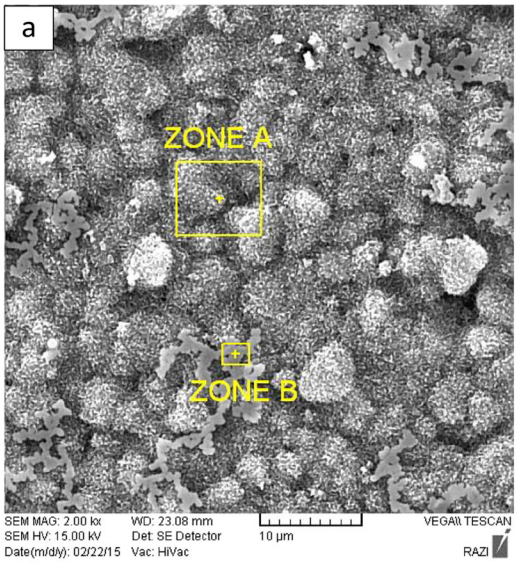


Element	Wt% in different zones	
	A	B
Carbon	11.54	16.50
Oxygen	42.96	39.81
Calcium	32.77	29.13
Bismuth	11.58	9.59
Iron	0.17	0.71
Silicon	0.43	3.28
Aluminium	0.22	0.48
Trace elements	0.33	0.5

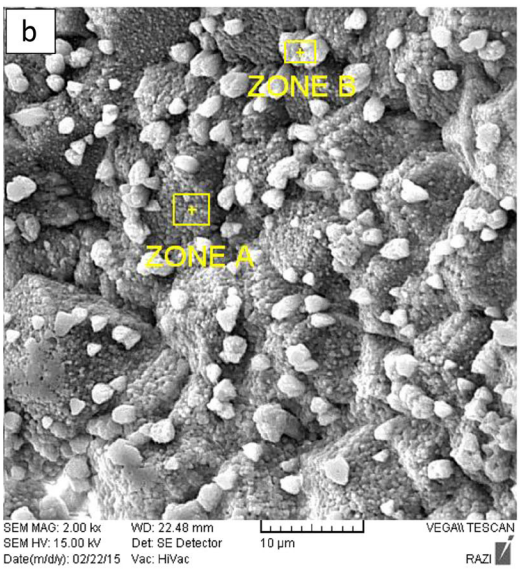


Element	Wt% in different zones		
	A	B	C
Carbon	10.80	6.09	9.64
Oxygen	45.17	37.82	44.76
Calcium	40.20	46.86	38.10
Bismuth	3.59	4.77	2.91
Silicon	-	4.40	4.20
Trace elements	0.24	0.06	0.39

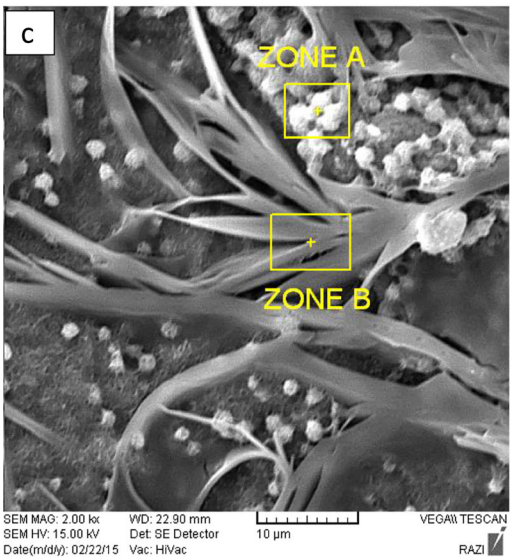
Fig. 1 Scanning electron microscopic (SEM) images and EDX results of ProRoot MTA exposed to (**a**) PBS, (**b**) blood, and (**c**) butyric acid showing globular crystalline structures in the PBS and blood exposed specimens and prismatic crystalline structures in those exposed to butyric acid



Element	Wt% in different zones	
	A	B
Carbon	5.34	5.99
Oxygen	37.69	39.79
Calcium	31.59	31.18
Zirconium	12.36	10.78
Chlorine	1.00	0.73
Silicon	9.72	8.95
Sodium	1.61	1.66
Trace elements	0.69	0.92

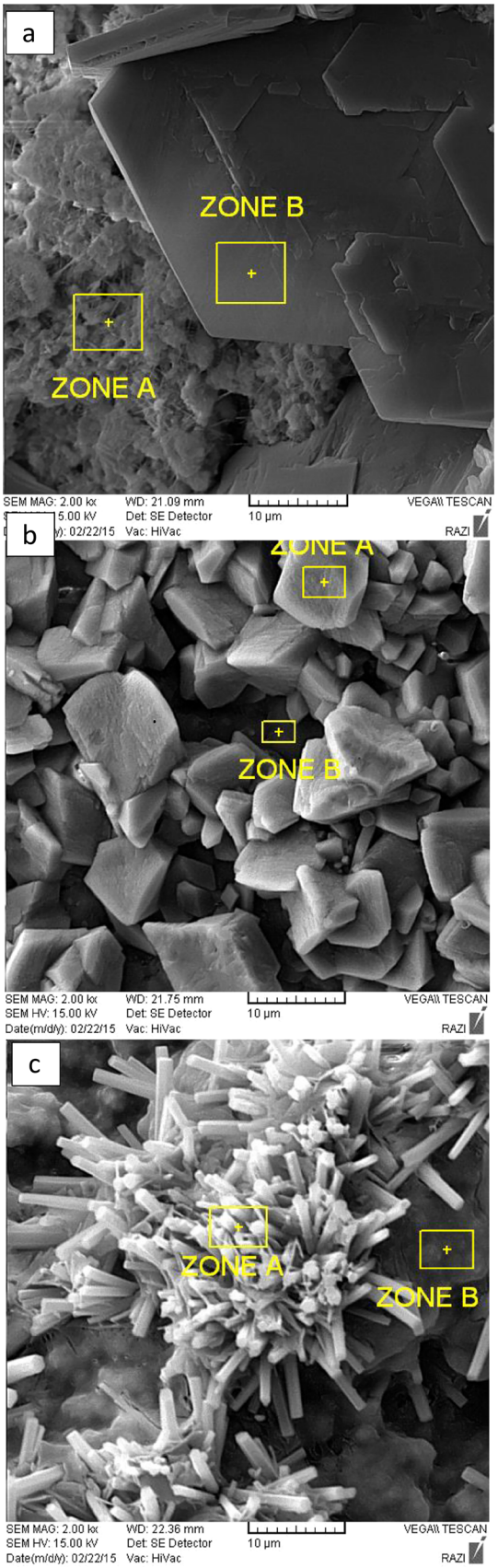


Element	Wt% in different zones	
	A	B
Carbon	9.16	11.81
Oxygen	26.13	36.94
Calcium	51.80	33.12
Zirconium	10.56	13.42
Chlorine	1.86	2.89
Silicon	-	0.87
Iron	-	0.54
Trace elements	0.49	0.41



Element	Wt% in different zones	
	A	B
Carbon	9.31	23.21
Oxygen	38.67	35.31
Calcium	40.07	32.44
Zirconium	9.10	6.59
Chlorine	1.26	0.53
Silicon	1.57	1.48
Trace elements	0.02	0.44

Fig. 2 Scanning electron microscopic (SEM) images and EDX results of Biodentine exposed to (**a**) PBS, (**b**) blood, and (**c**) butyric acid showing globular crystalline structures in all exposure conditions

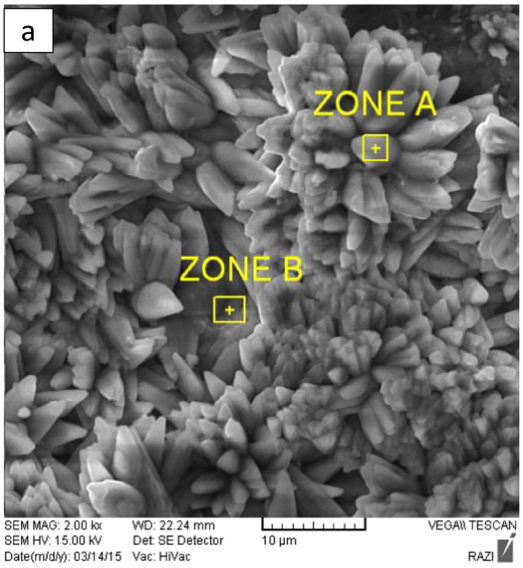


Element	Wt% in different zones	
	A	B
Carbon	-	1.41
Oxygen	26.58	41.43
Calcium	44.88	51.73
Silicon	1.86	-
Sulfur	4.74	0.92
Phosphorus	4.67	2.78
Zinc	4.73	-
Barium	12.14	1.38
Trace elements	0.4	0.35

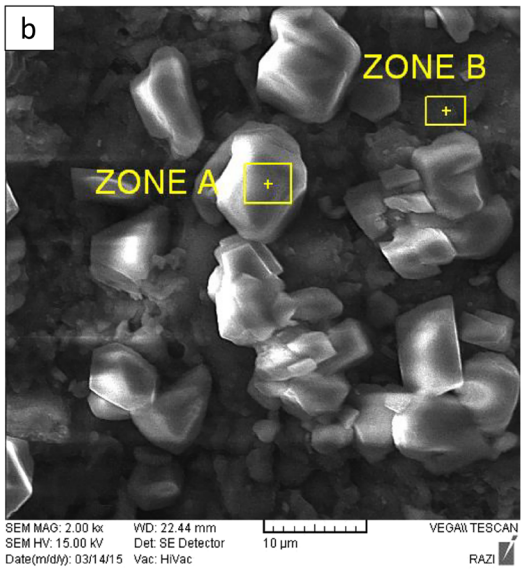
Element	Wt% in different zones	
	A	B
Carbon	10.50	0.50
Oxygen	41.39	8.46
Calcium	40.17	87.69
Sulfur	2.03	0.87
Phosphorus	5.49	0.88
Barium	-	1.60
Trace elements	0.42	-

Element	Wt% in different zones	
	A	B
Carbon	12.46	8.55
Oxygen	37.26	41.75
Calcium	27.43	33.02
Silicon	1.49	4.19
Sodium	0.44	2.65
Aluminium	4.14	0.67
Sulfur	3.14	-
Iron	0.76	-
Barium	12.49	-
Trace elements	0.39	9.17

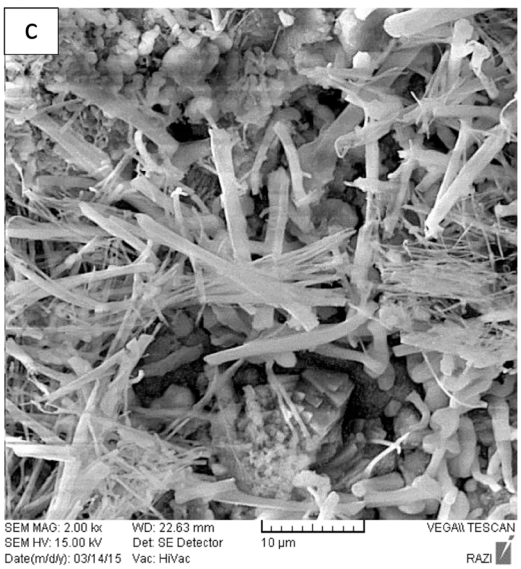
Fig. 3 Scanning electron microscopic (SEM) images and EDX results of CEM Cement exposed to (**a**) PBS, (**b**) blood, and (**c**) butyric acid showing hexagonal, cubical, and needle-like crystalline structures in specimens exposed to PBS, blood, and butyric acid, respectively



Element	Wt% in different zones	
	A	B
Carbon	11.86	8.72
Oxygen	46.93	43.07
Calcium	40.00	47.81
Barium	0.78	-
Trace elements	0.43	0.4



Element	Wt% in different zones	
	A	B
Carbon	9.09	13.25
Oxygen	44.48	37.58
Calcium	45.81	48.10
Barium	0.60	1.06
Trace elements	0.02	0.01



Element	Wt% in different zones	
	A	B
Carbon	10.31	6.46
Oxygen	46.61	42.60
Calcium	41.07	45.70
Barium	0.89	-
Aluminum	0.80	0.55
Silicon	-	4.67
Trace elements	0.32	0.02

Fig. 4 Scanning electron microscopic (SEM) images and EDX results of Retro MTA exposed to (**a**) PBS, (**b**) blood, and (**c**) butyric acid exhibiting prismatic, cubical, and needle-like crystalline structures in specimens exposed to PBS, blood, and butyric acid, respectively

Table 1 CSCs evaluated

Cement	Composition		Manufacturer
	Powder	Liquid	
ProRoot MTA	Tricalcium silicate, tricalcium aluminate, dicalcium silicate, bismuth oxide	Distilled water	Dentsply Sirona, Ballaigues, Switzerland
Biodentine	Tricalcium silicate, zirconium oxide, calcium carbonate	Calcium chloride, a water-soluble polymer	Septodont, Saint Maur des Fosses, France
CEM Cement	Calcium oxide, sulfur trioxide, phosphorous Pentoxide, silicon dioxide, aluminum trioxide, sodium oxide, magnesium oxide, chloride	Water-based liquid	Bionique, Tehran, Iran
Retro MTA	Calcium carbonate, silicon dioxide, aluminum oxide, calcium zirconia complex	Distilled water	BioMTA, Seoul, Republic of Korea

SEM images of ProRoot MTA specimens exposed to PBS and blood revealed the presence of globular crystalline structures which disappeared in those exposed to butyric acid. Instead, prismatic crystalline structures were seen on the surface of those exposed to butyric acid (Fig. 1). EDX evaluations revealed the addition of Fe in the composition of crystalline structures in specimens exposed to blood.

Biodentine specimens contained globular crystalline structures on their surface in all exposure conditions (Fig. 2). EDX evaluations revealed the presence of Fe in the composition of the crystalline structures of specimens exposed to blood while the amorphous phase in these specimens lacked both Fe and Si.

CEM Cement revealed hexagonal, cubical, and needle-like crystalline structures in specimens exposed to PBS, blood, and butyric acid, respectively (Fig. 3). EDX evaluations revealed the presence of P in both crystalline and amorphous structures in specimens exposed to PBS and blood whereas this element was not detectable in those exposed to butyric acid. BA was also detected in all specimens regardless of the exposure conditions.

Retro MTA exhibited prismatic, cubical, and needle-like crystalline structures in specimens exposed to PBS, blood, and butyric acid, respectively (Fig. 4). EDX evaluations revealed the presence BA in all specimens regardless of the exposure conditions.

XRD evaluation of ProRoot MTA exposed to different media revealed the presence of tricalcium silicate and dicalcium silicate, alpha bismuth oxide, and $Ca(OH)_2$ in all specimens. The peak of tricalcium silicate and dicalcium silicate was smaller than that of unhydrated powder. The peaks of $Ca(OH)_2$ on blood- and acid-exposed specimens were smaller than that of specimens exposed to PBS. The unhydrated powder did not contain $Ca(OH)_2$ (Fig. 5).

All Biodentine specimens exposed to different media contained tricalcium silicate and dicalcium silicate, calcium carbonate, zirconium oxide, and $Ca(OH)_2$. The peak of tricalcium silicate and dicalcium silicate was smaller than that seen in the unhydrated powder. The peak of tricalcium silicate and dicalcium silicate and $Ca(OH)_2$ seen in specimens exposed to blood were similar to that of PBS-exposed specimens. On the other hand, those exposed to acid exhibited smaller peaks of $Ca(OH)_2$. The acid-exposed specimens were also associated with smaller peaks of tricalcium silicate and dicalcium silicate in comparison to the blood-exposed specimens (Fig. 5).

XRD evaluation of CEM Cement exposed to the various media revealed the presence of tricalcium silicate and dicalcium silicate, calcium carbonate, barium sulfate, and $Ca(OH)_2$ in all specimens. The peak of tricalcium silicate and dicalcium silicate was smaller than that seen in unhydrated powder. The peak of $Ca(OH)_2$ on specimens exposed to blood and acid was smaller than when exposed to PBS (Fig. 5).

XRD evaluation of Retro MTA exposed to the media revealed the presence of tricalcium silicate, zirconium oxide, and $Ca(OH)_2$ in all specimens. The peak of tricalcium silicate was smaller than that seen in unhydrated powder. The peak of $Ca(OH)_2$ seen on specimens exposed to blood and acid was smaller than those exposed to PBS (Fig. 5).

Discussion

In this study, three exposure conditions were evaluated: exposure to PBS (pH = 7.4), WHB, and BA (pH = 5.4). BA is a short-chain fatty acids produced by endodontic pathogens and the most common fatty acid found in endodontic infectious conditions [22]. It is usually used to reproduce acidic conditions in vitro [19, 24]. PBS is a simulated tissue fluid containing phosphate [25] that can be used for the purpose of mimicking normal in vivo conditions in laboratory studies [19, 26].

Scanning electron microscopy and EDX analysis together with X-ray diffraction analysis were used to assess the effect of exposure to several potential clinically relevant

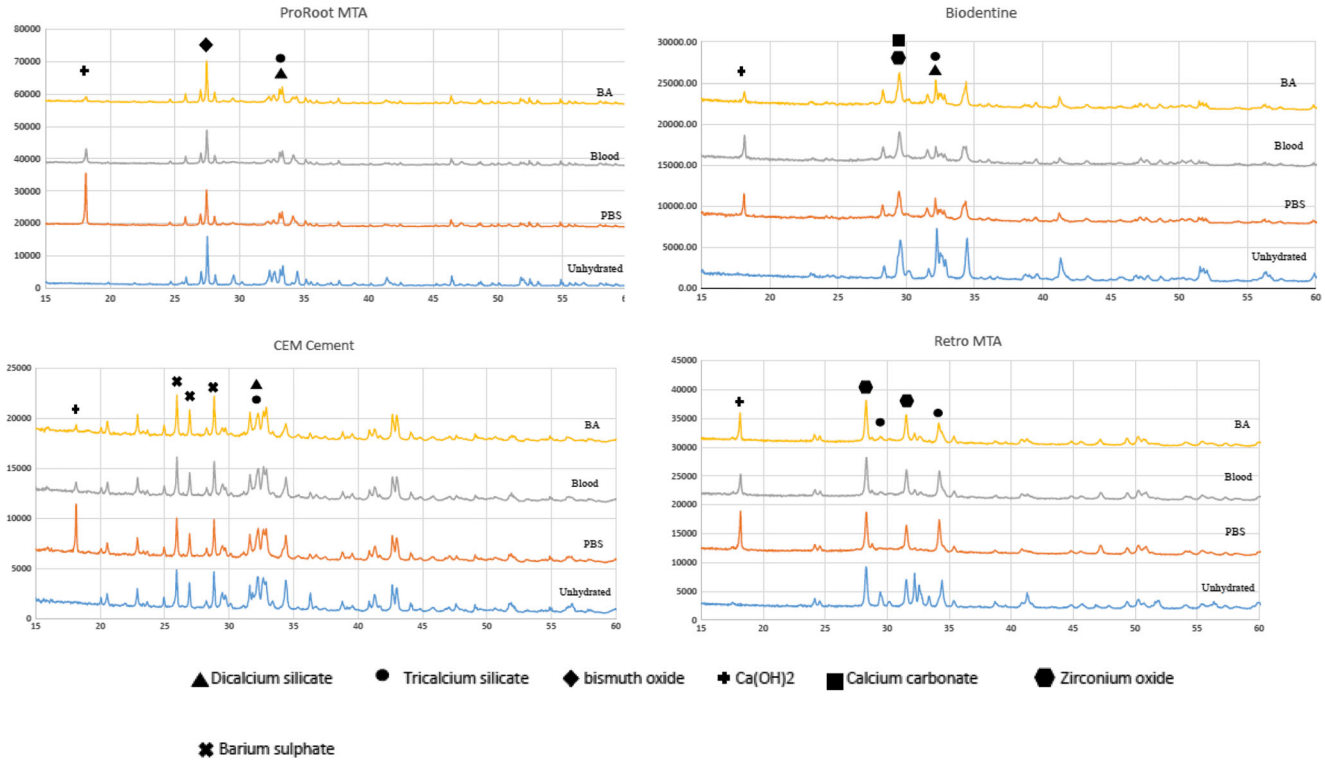


Fig. 5 X-ray diffraction result of different experimental groups

environmental conditions on the composition and hydration process of various CSCs.

XRD is useful for identifying the crystalline phases of specimens. The intensity of each peak representative of a crystalline phase in XRD is proportional to the phase concentration [27]. However, a disadvantage of this analysis for CSCs is the superimposition of the peaks and the presence of multiple compounds within the materials [27]. An example of this superimposition is observed at 52° 2θ for dicalcium silicate and tricalcium silicate crystalline structures as seen in Fig. 5. It should be noted that superimposition is also observed at 25.8° 2θ for hydroxyapatite and barium sulfate making them indistinguishable.

Reduction in the peak size of tricalcium silicate and dicalcium silicate peaks and detection of $Ca(OH)_2$ in the hydrated forms of all the CSCs in comparison with their powder counterparts is a consequence of the hydration of these CSCs [28–30]. Carbonation of $Ca(OH)_2$ should also be considered as it is inevitable in both clinical and experimental conditions but consistent in all specimens [31].

During the hydration process, calcium silicate hydrates (CSH) are also formed but due to their amorphous structure this phase was not detected with XRD analysis [27, 29, 30]. EDX analysis of the amorphous structures identified in SEM evaluations revealed the presence of Ca, Si, and O, which are indicative of CSH.

In ProRoot MTA, exposure to blood and acid reduced the peak size of $Ca(OH)_2$ compared to specimens exposed to PBS. These results are consistent with previous studies [20, 26, 32]. Two hypotheses can be attributed to this decrease: (a) prohibition of hydration due to exposure to different environmental conditions or (b) dissolution of $Ca(OH)_2$ formed as a consequence of hydration. Considering that the peak size of tricalcium silicate and dicalcium silicate was similar in all exposure conditions and smaller than that of the unhydrated powder, it may be concluded that the second hypothesis is more probable.

In the case of Biodentine, the peak size of $Ca(OH)_2$ in specimens exposed to PBS and blood were similar, however, in specimens exposed to acid a smaller peak was seen. Interestingly, the peak size of tricalcium and dicalcium silicate was seen in those exposed to acid was quite similar to the unhydrated powder indicating an impairment in the hydration process. Previous studies have reported that several physical properties of Biodentine were affected by acidic environmental pH [32] but not affected by exposure to blood [33]. This may be explained by the presence of calcium chloride [34, 35] and calcium carbonate that both accelerate the setting process [30] and the presence of higher percentage of amorphous phase in its hydrated form [36], thus making this cement less susceptible to environmental conditions. Further research is required regarding this matter.

In the case of CEM Cement, the peak of $Ca(OH)_2$ in specimens exposed to blood and acid was smaller than those exposed to PBS. However, their tricalcium and dicalcium silicate peaks were similar and smaller than those seen in the

unhydrated powder. Hence, it may be concluded that when CEM Cement is exposed to either blood or acidic pH, hydration occurs and $Ca(OH)_2$ is produced but is dissolved similar to what happens to ProRoot MTA. These conditions were also seen in Retro MTA.

Ettringite was not detectable in any of the specimens in the current study although trace amounts of Al were detected in EDX analysis of ProRoot MTA, CEM Cement, and Retro MTA. This may be due to either formation of small amounts of this phase [26] or limited detection due to dilution as a consequence of grinding the specimens [20]. The absence of ettringite peaks in the hydrated form of Biodentine was predictable as this cement lacks aluminate in its powder [30]. The reduction/absence of the aluminate phase is an added benefit with respect to the workability of the fresh cement paste [30], associated with biological improvements [37], e.g., avoiding the undesirable effects of aluminum (e.g., risks of Parkinson's and Alzheimer's disease) [38] and avoiding the leaching of this trace elements into the surrounding tissues [39, 40].

Conclusion

Blood or acidic pH exposure resulted in reduction in $Ca(OH)_2$ in ProRoot MTA, Retro MTA, and CEM Cement. Blood did not affect the hydration of Biodentine, whereas, acidic pH interfered with its hydration.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent For this type of study, formal consent is not required.

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