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Running title:

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Abstract

Objective: This study compares the physical properties and bioactivity of tricalcium silicate-based cement (Matreva MTA) modified with various concentrations of Aloe vera (AV) solutions and Biodentine cement.

Methods: Sixty discs were prepared, and divided into 5 groups (12 discs each) based on the estimated materials: group I: Biodentine, group II: Matreva MTA, group III: Matreva MTA with 10% AV, group IV: Matreva MTA with 20% AV and group V: Matreva MTA with 30% AV. The flow and setting time were assessed following ISO standard 6876:2012 and American Society for Testing and Materials (ASTM) standard C266-21, respectively. The in-vitro bioactivity was evaluated after 1, 14, and 28 days of immersion in Hank's balanced salt solution (HBSS) including pH, calcium ion release, and apatite formation. All data were statistically analyzed.

Results: Increasing the AV concentration added to Matreva MTA from 0 to 30% decreased the flow and setting time. Significant high mean pH and calcium ion release values were observed for 20 and 30% AV-modified Matreva MTA cement at 14 and 28 days ($P \leq 0.05$). Environmental scanning electron microscope/ energy-dispersive X-ray spectroscopy (ESEM/EDX) analysis confirmed calcium phosphate nucleation on the surfaces of 20 and 30% AV-modified Matreva MTA cements after immersion in HBSS.

Conclusion: Addition of 20 and 30% AV solutions to Matreva MTA reduced the setting time and improved the handling characteristics as well as the in-vitro bioactivity, resembling the qualities of Biodentine. Both AV-modified Matreva MTA and Biodentine cements had extended alkalinizing activity and calcium ion release.

Clinical relevance: AV-modified Matreva MTA can be considered a promising biomaterial for different endodontic applications.

Keywords:

Aloe vera, Bioactivity, Biodentine, Flow test, Matreva MTA, Setting time

Introduction

Mineral trioxide aggregate (MTA) has received considerable attention due to its outstanding biocompatibility, sealing ability, and antibacterial qualities [1-5]. It was first developed as a material for the repair of root perforations, but it is now utilized in regenerative endodontic therapy, vital pulp therapy, and the management of dentine hypersensitivity [6-9]. However, the practitioner is still faced with difficulties due to its lengthy setting time, challenging handling characteristics, expensive material cost, and discoloration potential [4,10].

Biodentine (Septodont, Saint Maur des Fosses, France), was recently introduced as a new fast-setting biomaterial that is suggested for use as a dentine replacement in similar applications as MTA [11-13]. The addition of setting accelerators and softeners, as well as a new pre-dosed capsule formulation for use in a mixing device, primarily improve the physical properties of biodentine, making it much more user-friendly [14,15].

Aloe vera plant belonging to the *Liliaceae* family has been widely utilized in pharmaceutical manufacturing due to its anti-inflammatory, antibacterial, antioxidant, antiviral, antifungal, hypoglycemic, immune-modulating action, wound healing, and regenerative properties [16,17]. *Aloe vera* powder contains more than 75 physiologically active and naturally occurring ingredients, including polysaccharides, amino acids, vitamins, lipids, sterols, and minerals [16]. Most of the functional activities associated with using *Aloe vera* plant are attributed to the polysaccharides (such as Acemannan and glucomannan) [16,18]. *Aloe vera* has been used to treat periodontal disease, angular cheilitis, aphthous ulcers, denture-related stomatitis, and intracanal medication [16,19]. When used as a pulp dressing material, AV has also proven to have anti-inflammatory and wound-healing properties [16,20,21].

Therefore, this study evaluated the physical properties and bioactivity of tricalcium silicate-based cement (Matreva MTA) modified with an AV-based solution in comparison with Biodentine for various endodontic applications.

Materials and methods

The materials utilized in the current study are shown in table 1.

Cement preparation and grouping of specimens

Matreva powder was mixed with either pure distilled water (0% AV) or with 10, 20, or 30% AV-modified distilled water at a powder/ liquid ratio of 3:1 by weight as recommended by the manufacturer. Biodentine was also mixed using the manufacturer's guidelines. The fresh pastes were packed into a split Teflon mold with a 10±0.1 mm diameter and 2±0.1 mm thickness on a clean glass slide. The top surface of the mold was then covered with a celluloid strip and a glass slide and left undisturbed until hardened (set). Sixty disc specimens were prepared for the five groups (12 discs each): group I: Biodentine, group II: Matreva MTA, group III Matreva MTA 10% AV, group IV: Matreva MTA 20% AV and group V: Matreva MTA 30% AV.

Assessment of the physical properties

Flow test

Each group's flow (n=6) was established in accordance with ISO standard 6876 [22]. After mixing, 50 μL (0.05 mL) of each substance was immediately placed onto a glass slab (40x40x5 mm). A second glass slab (20 g weight) was placed on the material with 100 gm weight on top. A digital caliper was then used to measure the maximum and minimum diameters of the formed disc and the mean diameter was considered as a measurement of the flow.

Setting time

The initial setting time and final setting time were measured (n=6 discs) by Gilmore needles in accordance with the ASTM standard C266-21 [23]. The initial Gilmore tip (113.4 g weight and 2.12 mm diameter) and the final Gilmore tip (453.6 g weight and 1.06 mm diameter) were utilized on the cement paste during the setting.

Bioactivity testing

Set cement discs from each investigated group (n=6 discs) were submerged in 10 mL of HBSS (Lonza, Walkersville, MD, USA) in a sealed 50 mL sterile polypropylene tube and kept in an incubator (Titanox, TITANOX art, A3-213-400I Co., Italy) at 37°C. The solution was collected for pH and calcium ion evaluation after 1, 14, and 28 days.

Alkalizing activity and calcium ion release

The pH was quantified using a pH meter (HI98103, Hanna Instruments Inc., USA). Three readings were average for each sample. Calcium ion analysis was analyzed using an inductively coupled plasma mass spectroscopy (ICP/MS) (Agilent 7700, Agilent Technologies, Germany).

Apatite-forming ability

The discs were collected after 1, 14, and 28 days. The specimens' surfaces were tested without any modification or coating using an ESEM attached to EDX Unit (Inspect, FEI Company, Netherlands) at 2000 and 5000 X. Elemental analysis using EDX was utilized to estimate the surface calcium-to-phosphorus (Ca/P) atomic ratios. The discs were returned into a new 10 mL of HBSS after 1 and 14 days and kept in the incubator until the following interval. The discs were scrapped with a sterile spatula to be examined by X-ray Diffraction (XRD) and Fourier-Transform Infrared (FTIR) spectroscopy after storage in the Hank's balanced salt solution (HBSS) for 28 days.

Statistical analysis

Data were analyzed using one-way ANOVA (setting time and flow test), and two-way ANOVA (pH and ion release test), followed by Tukey's post hoc test. The significance threshold was set at $P \leq 0.05$ for all tests. Statistical analysis was performed using the R statistical analysis software for Windows, version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Physical properties of the tested materials

Flow and setting time findings

Group II had the highest mean flow value while group V showed the lowest mean value (Table 2). There was a statistically significant difference among groups II and III ($P<0.05$) while no statistically significant difference was noted among groups I, IV and V ($P>0.05$).

Regarding the initial setting time, group II showed the highest mean value while group I showed the lowest mean value. The pairwise comparison revealed a significant difference among all groups ($P<0.05$) except for groups I and V, where no significant difference was found ($P>0.05$).

For the final setting time, group II showed the highest mean value while group I showed the lowest value. Pairwise comparison showed a significant difference among all groups ($P<0.001$).

Bioactivity of the tested materials

Alkalizing activity and calcium ion release results

The tested material, time, and their interaction had a significant effect on pH and Ca^{+2} ion release values (P<0.001).

Regarding the pH measurements (Figure 1a), day 1 showed the highest mean value followed by day 14 while day 28 showed the lowest mean value in all groups with a significant difference among the different time periods (P<0.001).

As for calcium ion release (Figure 1b), the highest mean value was observed on day 1 followed by day 14 while day 28 showed the lowest mean value in group I (P<0.001). On the other hand, in groups II, III, IV, and V, day 28 showed the highest mean value followed by day 14 while day 1 showed the lowest mean value with a significant difference among the different time periods (P<0.001).

Apatite forming ability

ESEM and EDX findings after immersion in HBSS

The results of ESEM of the Biodentine stored in HBSS for different periods showed the gradual formation of superficial spherules on the Biodentine surface. The amount of surface layer increased with increasing the immersion time from 1 d to 28 d. The EDX elemental analysis showed peaks denoting the different reaction phases present in the set cement and calcium phosphate layer with decreasing calcium/phosphorus ratio over time. These calcium phosphate crystals suggested the creation of an apatite layer (Figure 2). Increasing the soaking time in HBSS revealed the disappearance of zirconium peaks and a decrease in the intensity of silica components while Mg element from HBSS became detectable.

When Matreva MTA and AV-modified Matreva MTA groups were stored in HBSS for different time intervals; ESEM analysis revealed that superficial irregularly distributed Ca and P precipitates were gradually forming on the surface of the Matreva MTA (Figures 3-6). This surface layer grew when the immersion period was extended from 1 to 28 d. In comparison to the unmodified Matreva MTA during the same period, the calcium phosphate that was generated on the surface of the AV-modified groups was much denser. The calcium phosphate layer and other reaction phases were visible as peaks in the set cement's EDX measurement, and the calcium/phosphorus ratio decreased with time.

XRD findings after immersion in HBSS

XRD analysis of the hydrated Biodentine revealed peaks denoting calcium carbonate (calcite), and calcium hydroxide (portlandite) with obliteration of the tricalcium silicate peaks at the range of 32 to 40°. XRD spectral analysis of the hydrated Matreva MTA revealed peaks denoting calcium carbonate (calcite) at 30° and calcium hydroxide (portlandite) at 17°. According to the results of XRD, the calcium hydroxide peak increased after immersion in HBSS for 28 d. However, its peak slightly decreased with an increasing ratio of AV from 0 to 30% (Figure 7a).

FTIR findings after immersion in HBSS

After immersion in HBSS, FTIR analysis of Biodentine revealed distinct split peaks at ~500, 620, and 820 cm^{-1}. These peaks were consistent with apatite development representing apatite precursors, such as octa-calcium phosphate. A broad band of CSH within the 800–1000 cm^{-1} range was observed. Matreva MTA's FTIR absorbance spectra showed intensified bands of portlandite, CSH phase, and an apatite deposit. In addition, the bands of calcium carbonate (calcite, aragonite, vaterite), belite, and alite were still observable. A high band at ~950 cm^{-1} was assigned to PO_4^{3-} from apatite. The CO_3^{2-} stretching from calcite ($CaCO_3$) and carbonated apatite was visible at ~1400 cm^{-1}. Bands at ~1070 and 860 cm^{-1} were also assigned to PO_4^{3-} denoting symmetric stretching from apatite (Figure 7b).

Discussion

The current study evaluated the impact of AV solutions for the modification of tricalcium silicate-based cement (Matreva MTA), as a lower-cost alternative to Biodentine cement. Biodentine is known to have good physical properties, handling characteristics, biocompatibility, and wide clinical applications [13,24].

The flow and setting time were assessed following ISO standard 6876:2012 and ASTM standard C266-21, respectively. No significant differences in flow were found among Biodentine, 20% AV, and 30 % AV-modified groups. The modification of Matreva MTA with AV slightly reduced the flow and imparted the material with a putty-like consistency which may favor easier clinical manipulation. This was in accordance with a previous study by Borges et al. who investigated the physicochemical features of MTA and Portland cement associated with a 2% glycolic solution including *Aloe vera* [25]. Another study examined the physicochemical, antibacterial, and biological effects of white MTA combined with aqueous and propylene glycol extracts of *Arctium lappa* L. and *Casearia sylvestris* SW. The findings indicated that propylene glycol-mixed cement had long initial and final setting times and increased flow [26].

Regarding the setting time, the findings showed that the AV-modified Matreva MTA groups had a shorter setting time (initial and final) compared with the unmodified Matreva MTA. The mean initial setting time reduced from ~39.21 to ~17.83 min, and the mean final setting time decreased from ~98.67 to ~58.83 min, respectively, as the concentration of AV solution increased from 10 to 30%. While the initial setting times for the AV-modified Matreva MTA groups and Biodentine were comparable. A short setting time, like that obtained in the present study for 30 % AV-modified Matreva MTA is advantageous as it allows placement of the final restoration at the same visit. The ability of AV to create aggregated lumps of hydrophilic colloids may have accelerated the final set by facilitating the transport of ions and water into the calcium silicate [27]. Fortunately, this was not associated with a compromise in its workability.

The release of calcium and hydroxide ions from endodontic and pulp capping materials is considered a key factor for their success [28]. Calcium and hydroxide ions stimulate alkaline phosphatase enzymes and the differentiation of hard tissue-forming cells for mineralization/ bioactivity [29]. ICP-MS was used to measure calcium ion release because it is an advanced and accurate method analyzing of leached ions in media in trace levels despite the liquid pH [30].

Both Biodentine and Matreva MTA groups released calcium ions and induced alkalization (elevated pH). Biodentine initially released a significantly higher amount of calcium ions (253 ppm) after one day and produced a strong alkalization which significantly decreased with time in contrast to unmodified Matreva MTA which released the lowest calcium ion value (9.71 ppm). This may be due to the high solubility and fast hydration reaction of Biodentine in the early few hours after preparation. These findings are in accordance with earlier results [31]. This contrasted with prior research by Kumari et al. which found that MTA released considerably more Ca ions than Biodentine during the experiment but that difference increased and became greater after 28 d [32].

The pH measurements of all groups gradually decreased as the storage time increased from 1 to 28 d. This agreed with a previous study by Dsouza et al. [33]. However, calcium ion release significantly increased with increasing the storage time except for Biodentine, which decreased with time. These ions are then released to the surrounding medium where the hydroxyl ions increase pH which could induce an antibacterial effect and favors apatite precipitation [31,34]

The ion release process depends upon several factors like nature, size, porosity, density, and distribution of the mineral particles [3,34]. Therefore, porosity may increase with the increase in water sorption of AV-modified Matreva MTA providing a more bio-interactive surface for the calcium and hydroxyl ions release. This could explain the prolonged alkaline pH and the large, sustained calcium ions release observed in the present study.

In the current work, HBSS was used to simulate the ionic concentrations of the human blood plasma and mimic the biological fluids (blood, exudates, plasma, and dentinal fluid) as a source of phosphate ions to trigger the precipitation of calcium phosphate (apatite) on the surface of calcium leaching materials [35]. Thus, the experimental samples were soaked in HBSS for 28 d for the same specimen. This allowed a better correlation between the resultant data and monitoring of the changes that occur over time.

Different kinds of cements were evaluated for their *in-vitro* bioactivity using the ESEM/EDX, XRD, and FTIR analyses [3,13]. The utilization of the ESEM analysis was to avoid surface coating of the specimen before imaging, to prevent surface alterations, and to allow the usage of the same specimen throughout time intervals.

All materials formed superficial, spherical crystalline precipitates after being submerged in HBSS. These precipitates increased in amount and thickness from 1 to 28 d. These precipitates contained Ca and P as their main constituents, suggesting the formation of an apatite layer. EDX elemental analysis showed other elements in addition to the calcium phosphate layer. The Ca/P ratio decreased with increasing storage times as phosphorus peaks increased with increasing immersion time. ESEM assessment showed that AV-modified Matreva MTA produced a denser and thicker surface layer than the unmodified Matreva MTA cement at all-time intervals. The higher AV concentration also increased the precipitated calcium phosphate layer, suggesting the better apatite-forming ability of Matreva MTA cement. Biodentine and AV-modified Matreva MTA cement surfaces produced more precipitates, favored by the greater calcium ions release and higher pH of the surrounding media. This is consistent with another study's findings which revealed that alkaline salts greatly shortened the setting time, and increased ion release, and pH values of MTA and CEM cement [36].

Calcium phosphate deposition after immersion in HBSS indicates the amorphous or crystalline precursors of carbonated apatite or hydroxyapatite. The maturation of these precursors depends on the pH of the media and the time of immersion. The XRD analysis of the different groups after 28 days of immersion in HBSS revealed calcium hydroxide and calcium carbonate peaks. Peaks of crystalline hydroxyapatite were not detected, probably due to the low percent of the crystalline apatite formed. However, the results of FTIR spectra for the different groups after immersion in HBSS showed characteristic bands of phosphate groups due to the deposition of the apatite precursors on the material's surface. Similar findings were reported in earlier studies [37,38]. Moreover, Matreva MTA's *in vivo* bioactivity could be considerably enhanced by AV, generating moderate inflammation and good dentine bridge development comparable to Biodentine [12].

Conclusion

Addition of 20 and 30% AV solutions to Matreva MTA reduced the setting time and improved the handling characteristics as well as the *in-vitro* bioactivity, resembling the qualities of Biodentine. Both AV-modified Matreva MTA and Biodentine cements had extended alkalinizing activity and calcium ion release.

Clinical relevance

AV-modified Matreva MTA can be considered a promising biomaterial for different endodontic applications.

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Conflict of interests:

The authors declare no competing interests.

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Table 1: The materials utilized in the current study

Materials	Brand name	Manufacturer	Composition	Lot number
Tricalcium silicate-based cement	Biodentine	Septodont, Saint-Maur-des-Fossés, France.	**Powder:** Tri-calcium silicate, di-calcium silicate, calcium carbonate and oxide (fillers), iron oxide, and zirconium oxide (radiopacifier). **Liquid:** Calcium chloride (accelerator), hydrosoluble polymer	B25376
	Matreva MTA	Matreva Dental Labs, Egypt.	**Powder:** Tri-calcium silicate, di-calcium silicate, tricalcium aluminate, Sulphur trioxide, titanium oxide, and bismuth oxide (radiopacifier). **Liquid:** Distilled water	11002
Aloe vera extract	Aloe Fuzion	Global Health Center, LP. Houston, Texas, USA.	Organic inner leaf Aloe	150CC

Table 2: The mean ± SD of flow and setting time for the various tested groups.

Parameters	Group (I) Biodentine	Group (II) Matreva MTA	Group (III) Matreva MTA 10% AV	Group (IV) Matreva MTA 20% AV	Group (V) Matreva MTA 30% AV	P-value
Flow test (mm)	11.85± 0.19^{C}	12.98± 0.22^{A}	12.55± 0.35^{B}	11.82± 0.10^{C}	11.56± 0.15^{C}	**<0.001***
Initial setting (min)	16.75± 0.76^{D}	43.67± 2.34^{A}	39.21± 0.93^{B}	20.88± 0.77^{C}	17.83± 1.17^{D}	**<0.001***
Final setting (min)	28.92± 1.02^{E}	109.33±1. 75^{A}	98.67± 3.01^{B}	84.33± 3.39^{C}	58.83± 2.64^{D}	**<0.001***

Means with different superscript letters within the same row are statistically significantly different *: significant at $P \leq 0.05$.

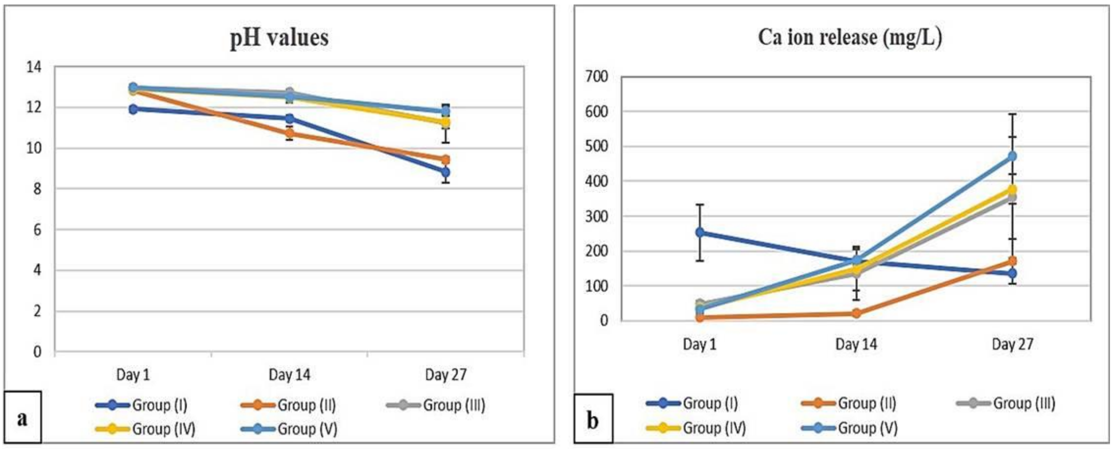
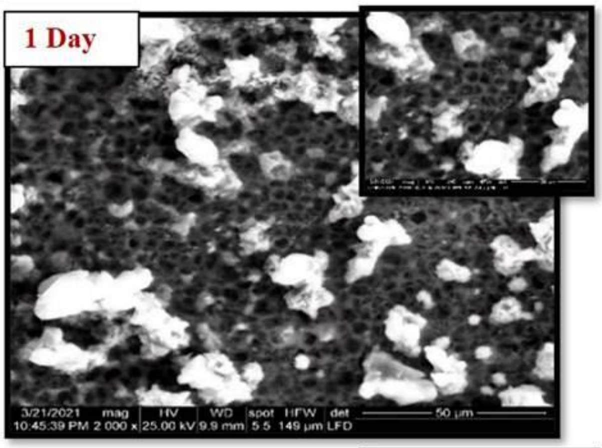
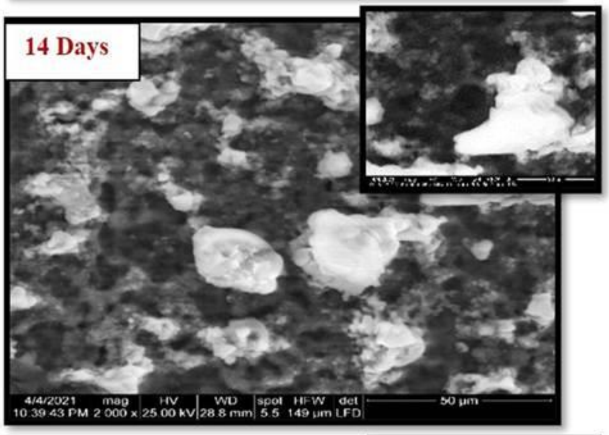


Fig. 1: Line chart showing **(a)** average pH values and **(b)** calcium ion release values (mg/L).



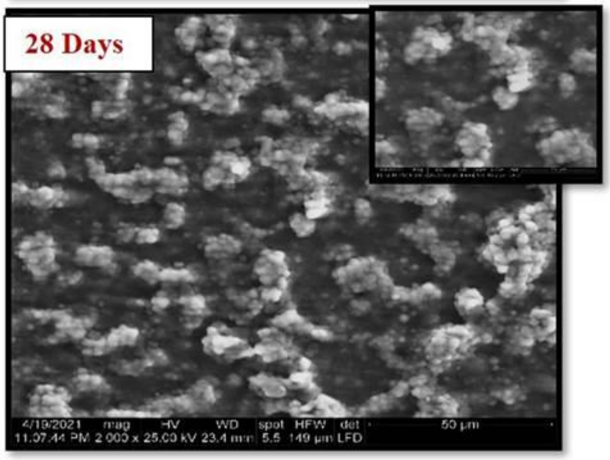
Element	*Wt %*	*At %*
C K	14.68	25.09
O K	39.28	50.42
SiK	03.21	02.35
P K	01.35	00.89
CaK	41.48	21.25

Ca/P = 23.87



Element	*Wt %*	*At %*
C K	10.11	17.98
O K	40.77	54.45
SiK	03.34	02.54
P K	03.99	02.75
CaK	41.79	22.28

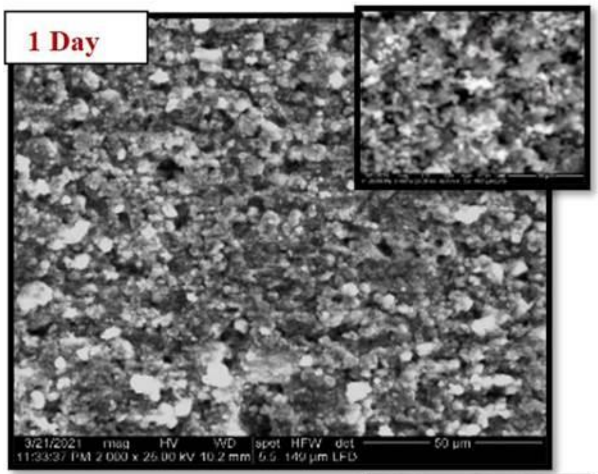
Ca/P = 8.10



Element	*Wt %*	*At %*
C K	08.63	16.08
O K	37.31	52.20
SiK	01.10	00.88
MgK	01.09	00.87
P K	07.67	05.54
CaK	38.60	21.11

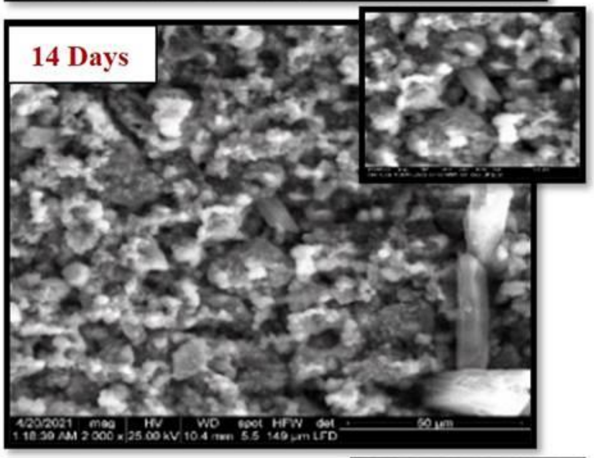
Ca/P = 3.99

Fig. 2: ESEM photomicrographs at different magnifications showing the surface deposits over the Biodentine disc stored in HBSS for different time intervals with the corresponding EDX analysis.



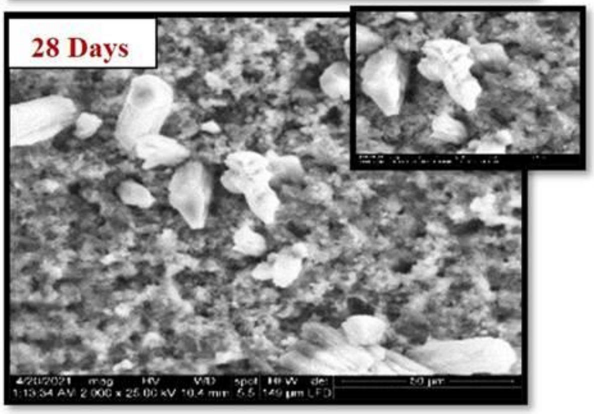
Element	Wt %	At %
C K	08.32	16.43
O K	37.45	55.50
NaK	01.52	01.57
AlK	00.57	00.50
SiK	04.06	03.42
P K	03.29	02.52
CaK	31.31	18.52
BiL	13.48	01.53

Ca/P = 7.35



Element	Wt %	At %
C K	09.66	18.62
O K	36.29	52.48
NaK	01.78	01.80
AlK	00.81	00.69
SiK	02.23	01.84
P K	04.93	03.68
CaK	34.27	19.79
BiL	10.02	01.11

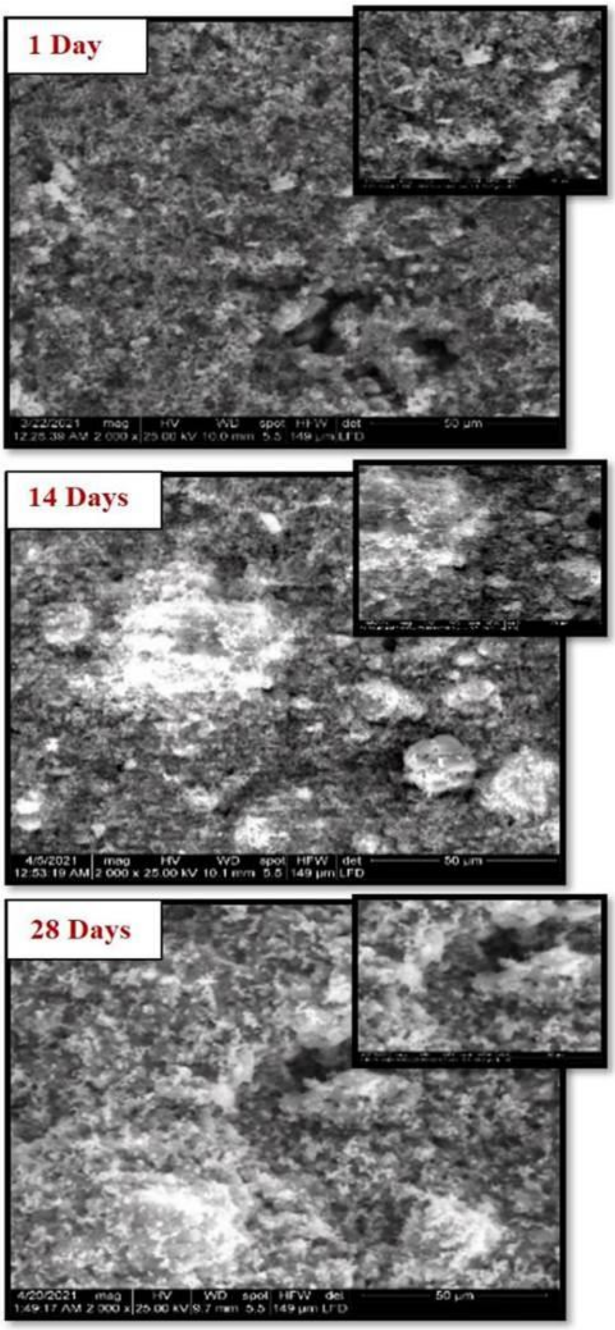
Ca/P = 5.38



Element	Wt %	At %
C K	06.54	12.69
O K	39.91	58.09
NaK	00.31	00.31
AlK	00.47	00.41
SiK	01.73	01.44
P K	08.95	06.73
CaK	33.32	19.36
BiL	08.77	00.98

Ca/P = 2.87

Fig. 3: ESEM photomicrographs at different magnifications showing the surface deposits over Matreva MTA disc stored in HBSS for different time intervals with the corresponding EDX analysis.



Element	Wt %	At %
C K	09.97	19.60
O K	35.89	52.96
NaK	01.62	01.66
AlK	00.92	00.80
SiK	04.12	03.46
P K	03.20	02.44
CaK	29.55	17.41
BiL	14.74	01.67

Ca/P = 7.13

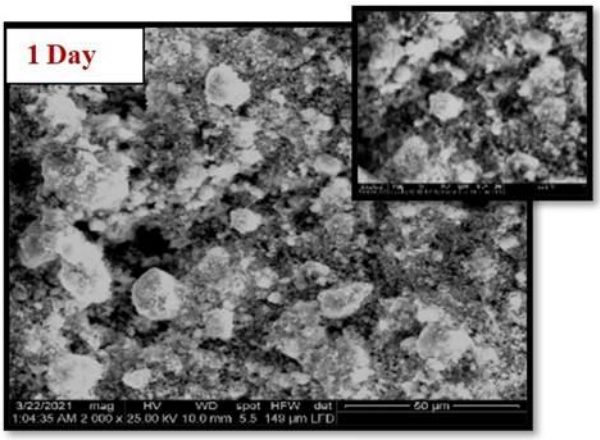
Element	Wt %	At %
C K	09.86	18.52
O K	39.24	55.30
NaK	02.30	02.26
AlK	00.65	00.54
SiK	01.67	01.34
P K	05.21	03.80
CaK	30.37	17.09
BiL	10.69	01.15

Ca/P = 4.49

Element	Wt %	At %
C K	06.60	12.57
O K	41.19	58.92
MgK	01.80	01.69
SiK	01.54	01.26
P K	09.35	06.91
CaK	31.02	17.71
BiM	08.51	00.93

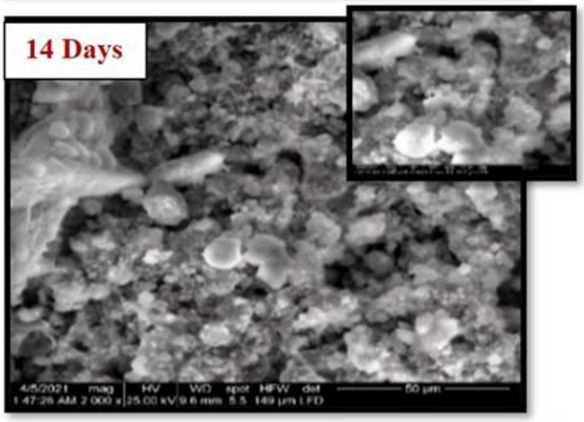
Ca/P = 2.56

Fig. 4: ESEM photomicrographs at different magnifications showing the surface deposits over Matreva MTA with 10% Aloe Vera disc stored in HBSS for different time intervals with the corresponding EDX analysis.



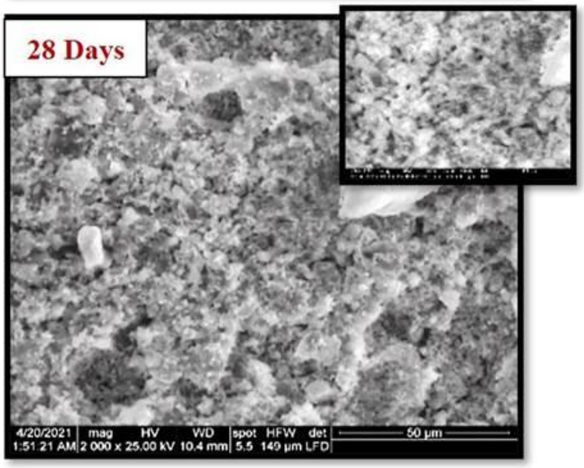
Element	Wt %	At %
C K	11.27	22.58
O K	34.29	51.60
NaK	01.04	01.09
SiK	03.91	03.36
P K	01.50	01.17
CaK	30.24	18.16
BiL	17.75	02.04

Ca/P = 15.52



Element	Wt %	At %
C K	12.12	22.75
O K	37.73	53.14
NaK	01.18	01.16
AlK	00.89	00.74
SiK	01.90	01.52
P K	04.17	03.04
CaK	28.89	16.24
BiL	13.11	01.41

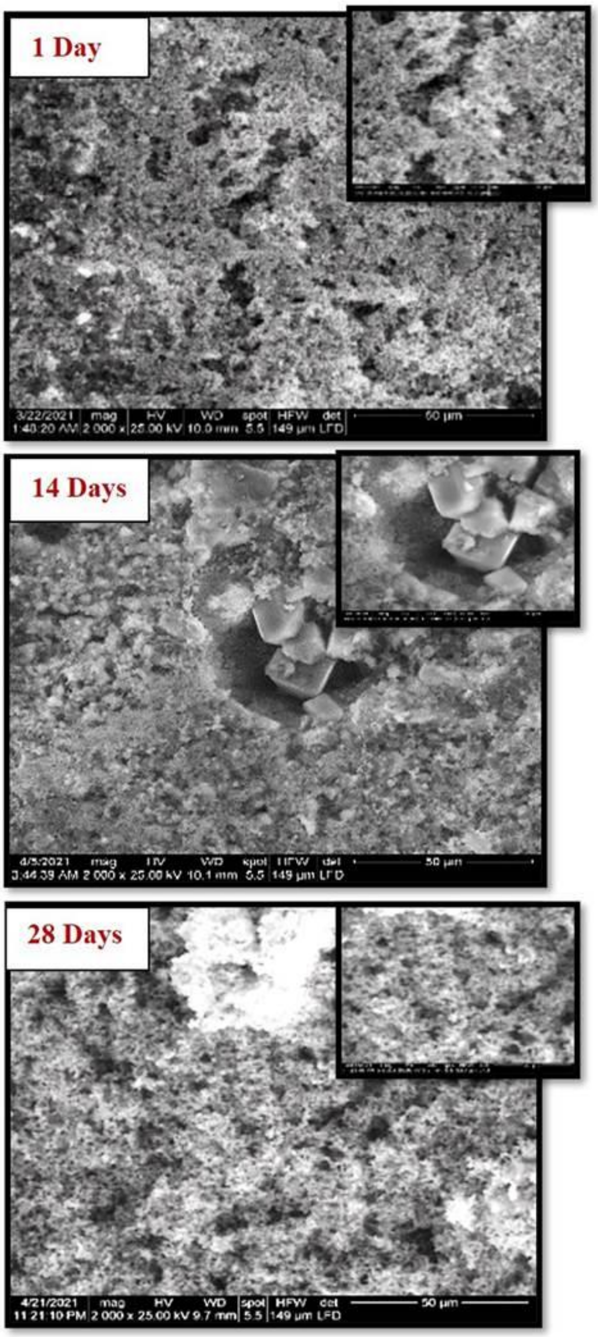
Ca/P = 5.34



Element	Wt %	At %
C K	09.44	17.75
O K	37.36	52.77
NaK	02.04	02.00
MgK	01.18	01.09
SiK	01.66	01.34
P K	08.48	06.19
CaK	31.92	18.00
BiM	07.93	00.86

Ca/P = 2.90

Fig. 5: ESEM photomicrographs at different magnifications showing the surface deposits over Matreva MTA with 20% Aloe Vera disc stored in HBSS for different time intervals with the corresponding EDX analysis.



Element	Wt %	At %
C K	13.41	25.66
O K	34.23	49.17
NaK	02.08	02.08
SiK	03.76	03.08
P K	01.38	01.02
CaK	28.78	16.50
BiL	15.42	01.70

Ca/P = 16.7

Element	Wt %	At %
O K	40.11	65.33
NaK	01.22	01.38
AlK	00.45	00.44
SiK	02.98	02.76
P K	06.21	05.23
CaK	35.68	23.20
BiL	13.35	01.66

Ca/P = 4.44

Element	Wt %	At %
C K	08.81	16.10
O K	40.59	55.66
MgK	01.29	01.17
SiK	01.74	01.36
P K	09.85	06.98
CaK	32.24	17.65
BiM	04.86	00.51

Ca/P = 2.52

Fig. 6: ESEM photomicrographs at different magnifications showing the surface deposits over Matreva MTA with 30% Aloe Vera disc stored in HBSS for different time intervals with the corresponding EDX analysis.

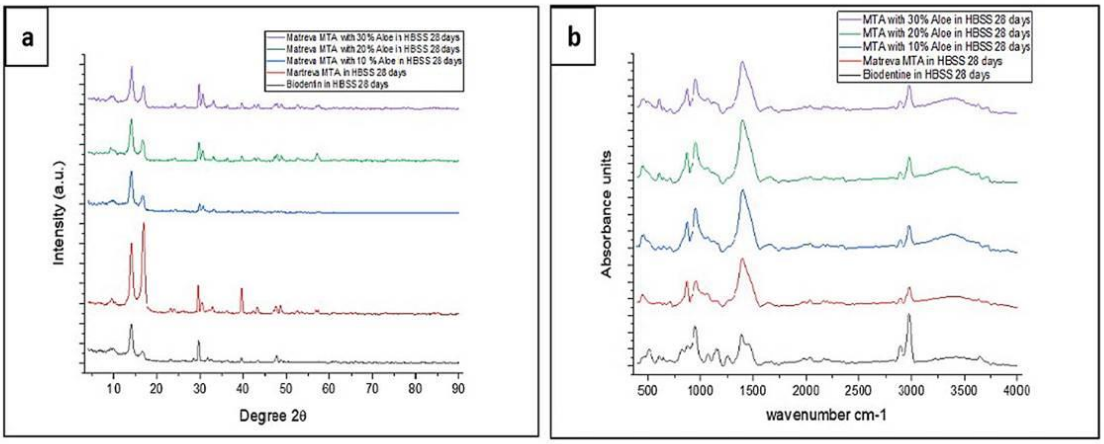


Figure 7: Images showing **(a)** XRD and **(b)** FTIR analysis of different groups after immersion in HBSS 28 days.