EGYPTIAN DENTAL JOURNAL
www.eda-egypt.org
VOL. 71, 3249:3266, OCTOBER, 2025
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
FIXED PROSTHODONTICS AND DENTAL MATERIALS
Submit Date : 11-07-2025 • Accept Date : 07-08-2025 • Available online: 01-10-2025 • DOI : 10.21608/edj.2025.402661.3547

EFFECT OF ADDING PHYTIC ACID ON SETTING TIME AND COMPRESSIVE STRENGTH OF MTA CEMENT VERSUS UNMODIFIED MTA: AN IN VITRO STUDY

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ABSTRACT

Aim: The present study aimed to evaluate the impact of Phytic acid addition to MTA on its setting time and compressive strength.

Materials and Methods: One hundred fifty-three discs of different mixtures of phytic acid and MTA were prepared and divided into three main groups (51 each). For each group, the following was measured: cell viability assay (n=3), bioactivity on three time intervals of 7, 14, and 28 days (n=3), calcium ion release at the same intervals of 7, 14, and 28 days (n=3), setting time (n=10), and compressive strength on two time intervals of 1 day and 7 days (n=10). Sulforhodamine-B assay was used to assess the cell viability. Bioactivity was measured using an environmental scanning electron microscope/energy-dispersive X-ray, and calcium ion release was measured using atomic absorption spectroscopy. Setting time was measured using the Gillmore apparatus, and a universal testing machine was used to measure the compressive strength.

Results: The results showed that a 2.5% aqueous solution of phytic acid showed higher cell viability. However, 5% phytic acid showed higher calcium ion release and more bioactivity. The results also demonstrated a statistically significant difference between the setting time of the control group and 2.5% and 5% aqueous solutions of phytic acid (p<0.001), with the control group exhibiting the highest mean and the 2.5% aqueous solution of phytic acid showing the lowest. Also, the compressive strength showed a statistically significant difference between the three test groups at the two time intervals. The highest compressive strength was found in the control group, while the 2.5% aqueous solution of phytic acid group showed the lowest.

Conclusions: Phytic acid had a great effect on shortening the setting time of MTA; however, there was an inverse effect on the compressive strength of MTA.

KEYWORDS: Phytic acid, setting time, MTA, compressive strength

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INTRODUCTION

Bioceramics are biomaterials successfully developed for use in the medical and dental fields. Bioceramics are inorganic, non-metallic materials with mechanical properties similar to the hard tissues they repair or replace. They are biocompatible, chemically stable, non-toxic, and non-corrosive materials that interact with the surrounding organic tissue**. (Dong and Xu 2023)**

Bioceramics used in endodontics are usually bioactive, with calcium silicate-based cement being the most widely used. Mineral trioxide aggregate (MTA) is the earliest developed and the most widely used bioactive calcium silicate-based ceramic material in dentistry. **(Tomer et al. 2020)** MTA is considered the gold standard for many clinical situations and is close to the ideal reparative material. MTA is a highly effective dental material that has improved the prognosis of bad clinical conditions. MTA is composed primarily of Portland cement with the addition of bismuth oxide, which makes MTA radiopaque. **(Pushpalatha et al. 2022)**

The clinical applications of MTA have expanded beyond its original uses. This is due to its biocompatibility, excellent sealing ability, and ability to form hydroxyapatite. MTA also shows favourable healing traits, as there is no inflammation or ankylosis. **(YÜZER, Güney Mustafa 2021)** MTA also simulates cytokine release, which promotes hard tissue formation. Also, MTA simulates bone healing, and this decreases the clinical symptoms. **(Malhan and Gobindgarh 2021)** This is in addition to its high alkalinity, which causes the release of calcium ions present in body fluid, facilitating mineralization and hard tissue formation. The high pH makes it a bacteriostatic material. MTA became a replacement for $Ca(OH)_2$, and research proved the superiority of MTA over $Ca(OH)_2$. MTA produces hydroxyapatite similar in composition and structure to natural hydroxyapatite**.(Camilo do Carmo Monteiro et al. 2017)**

Although MTA has a wide range of clinical applications, clinical use of MTA is limited by the long setting time and the fact that it can only be used in low-stress-bearing areas. Grey MTA requires about 2 hours and 55 minutes to set, while white MTA sets in 2 hours and 20 minutes. Therefore, due to the prolonged setting time of MTA, it should not be placed in single-visit treatments. **(Chen et al. 2018)**

Additionally, MTA's granular form and susceptibility to being washed away when exposed to blood or other fluids make it difficult to handle during clinical procedures. Also, the freshly mixed cement's gritty or sandy-like consistency complicates its placement and distribution to the surgical site.**(Pushpalatha et al. 2022)** MTA exhibited compressive strength of 40 MPa after 24 hours, which increased to 67.3 MPa after 21 days. **(Madhumitha Jayakumar, Shashikala K, and H Murali Rao 2018)**

According to the studies, it has been demonstrated that the setting time and compressive strength can be influenced by mixing with various liquids and additives. Different solutions can be used instead of distilled water; however, the material's physical properties might be compromised. Sodium hypochlorite gel and CaCl2 can shorten the setting time; however, their incorporation leads to a reduction in compressive strength when compared to MTA prepared with sterile distilled water. t**(YÜZER, Güney Mustafa 2021)** It is important to ensure that the mixing liquid contains sufficient water with the necessary diffusion ability to support the hydration process.

Phytic acid, also known as inositol hexaphosphate (IP6), is an antioxidant with an effect on preventing free radical formation. It has a high concentration of negative charges due to the presence of six phosphate groups, which become partially ionized at physiological pH. **(Dharmalingam et al. 2023**) Research on IP6 in dentistry started decades ago,

showing cariostatic, anti-cariogenic properties by lowering enamel solubility or binding to hydroxyapatite, which reduces bacterial adhesion to the tooth. It also has an anti-calculus and anti-plaque effect; this is besides its cement-forming properties and its ability to decrease enamel dissolution**. (Nassar et al. 2021)**

It has been proven that adding IP6 to dental cement shows improvement in both the chemical and physical properties of dental cements. IP6 was added to aluminosilicate glass, where it decreased its setting time. It was also added to zinc phosphate cement, which showed improvements in its setting time and mechanical properties. IP6 can accelerate the setting of calcium silicate-based cement(CSC) while maintaining its strength. **(Nassar et al. 2021)**

The impact of various concentrations of phytic acid on MTA has not been studied yet. So, this study aimed to assess the effect of 2.5% and 5% aqueous solutions of phytic acid after mixing with MTA. This study also aimed to study their effect on MTA setting time and compressive strength. The null hypothesis tested was that there would be no difference in the setting time and compressive strength between MTA mixed with 2.5% and 5% aqueous solutions of phytic acid and unmodified MTA.

MATERIALS AND METHODS

Materials

The materials utilized in this study are detailed in **Table 1.**

Methods

Sample preparation:

According to a pilot study done on the cell viability of MTA mixed with 70% phytic acid ($C_6H_{18}O_{24}P_6$) from Solarbio Science and Technology Co., LTD. (Beijing, China), it was diluted to get two different concentrations of phytic acid. A 5% aqueous phytic acid solution was obtained after adding 93 mL of distilled water to 7 mL of phytic acid. A 2.5% aqueous phytic acid solution was obtained by adding 96.5 mL of distilled water to 3.5 mL of phytic acid. This was conducted in the Animal Health Research Centre (Dokki, Cairo). A powder-to-liquid ratio of 3:1 was used to mix MTA with the distilled water or phytic acid until a putty-like consistency was obtained.

Three main groups of MTA were prepared as follows:

i) MTA mixed with 2.5% aqueous solution of phytic acid (Group A)

ii) MTA mixed with 5% aqueous solution of phytic acid (Group B)

iii) MTA mixed with distilled water (Control group)

TABLE (1) The brands used, composition, manufacturer, and batch number

Material	Composition	Manufacturer	Batch No.
MTA Cerkabiomed	Tricalcium silicate, Dicalcium silicate, Tricalcium aluminate	Cerkabiomed (Poland)	0201241
70% phytic acid	Inositol hexaphosphate	Solarbio Life Science (China)	201800604
Simulated body fluid	Solution of calcium and phosphate ions Salts of NaCl, $NaHCO_3$, KCl, $K_2HPO_43H_2O$, $MgCl_26H_2O$, $CaCl_2$, Na_2SO_4, and $(HOCH_2)_3CNH_2$	Faculty of Pharmacy using	
Distilled water	100 % Purified water	Faculty of Pharmacy	
Floss	Nylon	Oral-B/America	

Sample grouping

A total of one hundred fifty-three discs were fabricated in different sizes according to the test used in the study.

The discs were divided according to the following: nine discs of Teflon split mold were fabricated, 3 mm thick and 3 mm in diameter, to study the cell viability assay (n=3). Twenty-seven discs were fabricated with a diameter of 8 mm and 1.6 mm to study the bioactivity at three time intervals (n=3). Twenty-seven discs were fabricated with a diameter of 1 mm and a height of 1 cm for the calcium ion release test at three time intervals (n=3).

Thirty discs were fabricated of split Teflon with a mold size of 10 mm height and 2 mm diameter. (n=10) And finally, to study the compressive strength at two time intervals, sixty cylindrical discs were fabricated of stainless-steel mold, with dimensions of 3 mm diameter and 6 mm height (n=10).

Cell viability assay (SRB) (%):

The cell viability was evaluated using the Sulforhodamine-B (SRB), and the test was conducted based on the methodology demonstrated by **(Jaita et al. 2024), (Mathews, Hegde, and Thakur 2015),** and **(Alsofi 2019).** MTA was mixed with the aqueous solution of phytic acid and placed in Teflon split molds 3 mm thick and 3 mm in diameter under aseptic conditions and allowed to set for 24 hours at 37°C in a fully humidified environment (100% humidity). Following its setting, discs were exposed to ultraviolet light (UV) for sterilization for 20 minutes at Nawah Scientific Inc**.** (Mokattam, Cairo). Fresh MTA extract was obtained by immersing MTA in 1 mL of Dulbecco's modified Eagle medium (DMEM) per well for one day. The MTA extract would be used in the cytotoxicity test.

Human skin fibroblasts were sourced from Nawah Scientific Inc. The cells were cultured to form a monolayer in DMEM medium supplemented with 100 μg/mL streptomycin, 100 U/mL penicillin, and 10% heat-inactivated fetal bovine serum. Incubation was carried out at 37°C in a humidified atmosphere containing 5% (v/v) CO_2.

Then the adherent cells were detached with 2-3 ml of 0.05% trypsin and then incubated for 2-5 minutes at 37°C. The MTA extract was then separated from MTA material, and it was incubated close to the growing cell culture for 72 hours, and the cell number was examined using the SRB assay.

After 72 hours of exposure to MTA extract, cells were fixed using 150 µL of trichloroacetic acid and incubated at 4°C for one hour. Following the removal of the trichloroacetic acid, distilled water was used to wash the cells 5 times.

After being washed, the cells were exposed to 70 µL of SRB 0.4% (w/v) in 1% acetic acid solution and incubated for 10 minutes at room temperature in a dark room. Following three rounds of washing with a 1% acetic acid solution, the plates were left to dry. The protein-bound SRB stain was solubilized by adding 150 µL of unbuffered Tris. Then, a BMF LABTECH®-FLUOstar Omega microplate reader (Ortenberg, Germany) was used to measure the absorbance at 540 nm.

Bioactivity (ESEM and EDX)

Three samples per group (diameter 8.0 mm ± 0.1 mm, height 1.6 ± 0.1) were fabricated and were vertically immersed in 20 ml SBF, with one end of a thread of unwaxed dental floss embedded at the periphery of the sample during setting to facilitate handling. The discs were suspended in the SBF hanging from the lids of the glass tube, ensuring that their surfaces were free from contact with the tube walls and were exclusively exposed to the SBF. The discs were stored at 37°C for 7, 14, and 28 days. **(Figure 1)** Simulated body fluid was prepared following the protocol established by Kokubo and Takadama in the Faculty of Pharmacy (Manial, Cairo). This was done using NaCl, NaHCO3, KCl, K2HPO43H2O, MgCl26H2O, CaCl2, Na2SO4, and (HOCH2) 3CNH2.**(Waly and Salama 2018), (Elkhashab et al. 2023)**

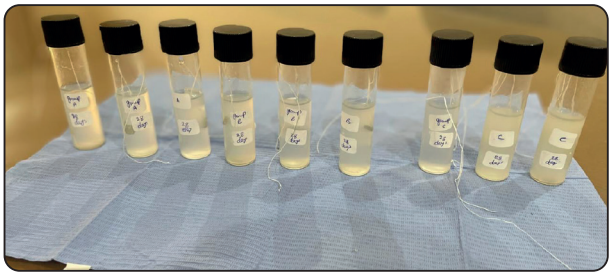

Fig. (1) Bioactivity samples suspended in SBF

The medium was renewed weekly with fresh SBF. At each endpoint, the samples were taken from the SBF, cleaned using distilled water, and examined.

An environmental scanning electron microscope (ESEM) (Model FEI Quanta 3D 200i) with energy dispersive x-ray analyses (EDX)/Thermofisher Pathfinder was used to analyze the surface of the moist sample at the Grand Egyptian Museum Conservation Centre (Giza, Egypt). To determine the superficial (Ca/P) atomic ratio, EDX offers semi-quantitative and qualitative measurements of calcium and phosphorus. Without any prior treatment, the samples were evaluated in moist conditions after being mounted on aluminum stubs using double-sided adhesive tape, and examined under an accelerating voltage of 20 or 30kV and magnification 3000 x, to identify the precipitates on the cement surface.**(Elkhashab et al. 2023)**

Calcium Ion Release Test:

Three samples per group were fabricated with a 1 mm internal diameter and 1 cm height in polypropylene tubes to measure calcium ion release. MTA paste was packed inside the polypropylene tubes using hand pluggers. Excess cement was removed from the two ends of the tube. Then the specimen was transferred to glass flasks containing 10 mL of deionized water. Then the flasks were kept at 37°C and 100% humidity in an incubator(BTC BT1120/Taiwan). And the amount of calcium ions released was measured using an atomic absorption spectrophotometer (Analytikjena) in the Agriculture Research Centre (Giza, Egypt) after 7 days, 14 days, and 28 days. **(Zanjani et al., 2018)**

Setting Time Test:

According to the American National Standard Institute/American Dental Association (ANSI/ ADA) No. 57 (200), ISO standard 6876-16, and the American Society for Testing and Materials (ASTM) international standard C266-08 (2008), ten specimens of each tested mix were prepared. The tested material was manually mixed at the specified P/L ratio on a dry, clean glass slab before being packed in a split Teflon mold with dimensions of 10+0.1 mm in diameter and 2+0.1 mm in height. **(Figure 2)**

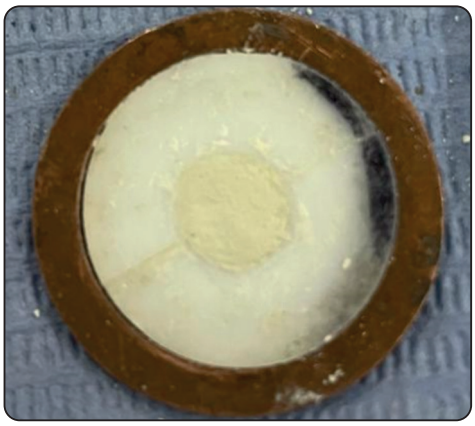

Fig. (2) MTA set cement in mold measuring (2mm*10 mm)

The Gilmore test instrument used for setting time assessment has two needles: the needle for testing the initial setting time weighed 113.4 g and had a tip of 2.12 mm in diameter, and the final setting time was measured using a weight of 453.6 g and a tip measured with a diameter of 1.06 mm**. (Figure 3)** After two minutes of mixing, the indenter was carefully and gently applied to the surface of the sample. The mix was considered initially set when the needle was lightly placed on the surface and failed to penetrate the material after 5 seconds. The load was then applied at 60-second intervals until no visible indentation was detected using the

1.06 mm needle. For each sample, the time between the start of mixing and the point at which no visible indentation was recorded was defined as the final setting time.**(Dianat et al. 2019), (Jamali Zavare et al. 2020)**

Compressive strength evaluation:

Ten samples from each group were placed in a stainless steel mold with a diameter of 3±0.1 and a height of 6±0.1 were prepared according to ISO 6876. All the samples were mixed by the same operator, and the mixing time was 10 seconds for all the samples. Within two minutes of mixing, the material was placed in the stainless steel mold and packed with an appropriate plugger to ensure a dense, uniform sample with minimal porosity. Vaseline was applied to the mould's interior surface. And excess material was removed using another glass slab to leave a flat, uniform surface. Then the samples were kept in distilled water and placed in an incubator (BTC BT1120/Taiwan) at 37°C in a humid environment. Samples were taken out of the molds and checked for any voids or chipped edges. All defective samples were discarded. The specimen's new dimensions were remeasured using a caliper. Samples were incubated for 1 and 7 days. Compressive strength was measured using a universal testing machine (Shimadzu AG-X Autography, Kyoto, Japan). after 1 day and 7 days. **(Figure 4)**.

The specimen was positioned horizontally, and a compressive load was applied at a crosshead speed of 1 mm/min, aligned parallel to the longitudinal axis of the mold. **(Tabari et al. 2017)** Then, based on the following equation, the compressive strength was calculated in megapascals (MPa):

$$CS = \frac{4P}{\pi D2}$$

where π is the constant 3.14, D is the specimen's diameter in millimeters, and P is the maximum load in Newtons. **(Eskandarinezhad et al. 2020)**

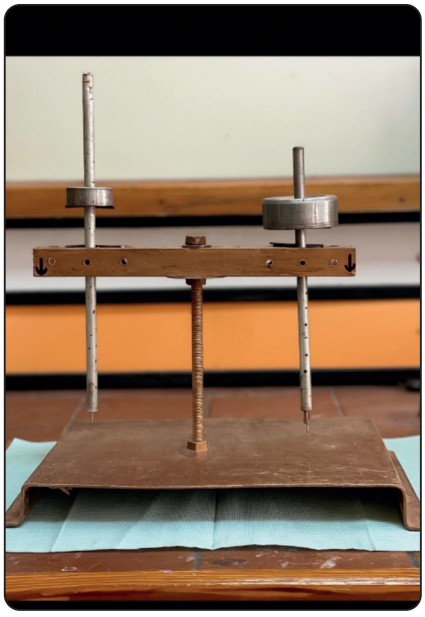

Fig. (3) Gilmore test instrument for measuring initial and final setting time

Fig. (4) Universal testing machine with the specimen aligned horizontally

Statistical analysis

For each group across all tests, the mean and standard deviation were determined. Data normality was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests, confirming a parametric (normal) distribution.

In non-related samples, more than two groups were compared using a one-way ANOVA followed by the Tukey post hoc test. Two groups in related samples were compared using the paired sample t-test. To assess the different parameters' interactions, a two-way ANOVA was used.

A significance level of $P \leq 0.05$ was set. IBM® SPSS® Statistics Version 25 for Windows was used to conduct the statistical analysis.

RESULTS

Cell Viability (SRB) %

The mean and SD values of the cell viability % for group A, group B, and the control group were (79.34±2.24), (71.86+0.43), and (75.83±2.55), respectively. Therefore, cell viability for the testing groups was as follows, from the highest to the lowest: group A, followed by the control group, followed by group B. **(Table 2)**

Bioactivity

7 days

After 7 days, the ESEM of group B revealed some spherical aggregates and needle-like crystals dispersed. High peaks of Ca, P, and Si indicated the presence of calcium phosphate deposits with a Ca/P ratio of 3.4. (**Figure 6)** However, the ESEM of group A, showed less spherical aggregates dispersed with fewer needle crystals, with a Ca/P ratio of 3.3. **(Figure 5)** Meanwhile, in the control group, only spherical crystals were found with no needle crystals dispersed, with a Ca/P ratio of 3.4 **(Figure 7)**

14 days

After 14 days, group B showed clusters of aggregates randomly distributed, with more defined hydroxyapatite crystals formed, with the Ca/P ratio being 1.9. **(Figure 6)**

In comparison, group A showed fewer hydroxyapatite crystals within aggregates, dispersed with a Ca/P ratio of 2.05. **(Figure 5)**

However, the control group showed well-arranged immature calcium phosphate clusters, with no crystals formed, with a Ca/P ratio of 3.01. **(Figure 7)**

28 days

After 28 days, group B showed spherical clusters, and the well-defined needle-shaped crystals became more intense with a high Ca/P ratio of 1.62. **(Figure 6)**

Group A exhibited a lower amount of needle-shaped crystals, accompanied by more abundant spherical aggregates, and a Ca/P ratio of 1.45. **(Figure 5)**

In contrast, the control group exhibited a very poor mineral deposition, with few needle-shaped crystals with a Ca/P ratio of 1.81. **(Figure 7)**

Ca ion release

7 days

The mean and SD values of the calcium ion release test for group A, group B, and the control group after 7 days in mg/L were (11.17±0.81), (10.92±1.23), and (7.72±0.67). After 7 days, the order of the calcium ion release for the different groups was as follows, from the lowest to the highest: the control group, followed by group B, and finally group A.

14 days

The mean and SD values of the calcium ion release after 14 days of immersion in deionized water for group A, group B, and the control group after 14 days were (14.25±0.70), (16.67±1.27), and (10.73±2.46). After 14 days, the calcium ion release exhibited the following order, from the lowest to the

highest: the control group, followed by group A, and lastly, group B.

28 days

The mean and SD values of the calcium ion release test for group A, group B and the control group after 28 days in mg/L were (15.99±0.59), (24.27±0.61), and (13.85±0.27). After 28 days, the calcium ion release showed a progressive increase in the following order: the control group, then group A, and finally group B. **(Table 3)**

Setting time:

Initial setting time:

The mean and SD values of the initial setting time in minutes for group A, group B, and the control group were (5.95±1.22), (8.75±0.72), and (34.61±2.28), respectively. One-way ANOVA showed a statistically significant difference between group A, group B, and the control group(p<0.001). The shortest setting time was found in group A, followed by group B, and finally, the longest setting time was found in the control group. The Tukey post-hoc test revealed a statistically significant difference between group A and each of group B and the control group, where (p=0.001) and (p<0.001). Also, there is a statistically significant difference between group B and the control group (p<0.001).

Final setting time:

The mean and SD values of the final setting time in minutes for group A, group B, and the control group were (21.01±1.10), (27.02±1.86), and (92.31±2.47), respectively. One-way ANOVA showed a statistically significant difference between group A, group B, and the control group (p<0.001). Group A showed the shortest setting time, followed by group B, and finally, the control group showed the longest setting time. The Tukey post-hoc test revealed a statistically significant difference between group A and each of group B and the control group, where (p<0.001). Also, there is a statistically significant difference between group B and the control group (p<0.001). **(Table 4) (Figure 8)**

Compressive strength

Day 1:

The mean and SD values of the compressive strength for group A, group B, and the control group after 1 day in MPa were (26.75±0.92), (31.32±1.38), and (33.63±1.98), respectively. One-way ANOVA showed a statistically significant difference between group A, group B and the control group (p<0.001). The lowest compressive strength value was found in group A, followed by group B, and the highest compressive strength was found in the control group. The Tukey post-hoc test revealed a statistically significant difference between group A and each of group B and the control group, where (p<0.001). Also, there is a statistically significant difference between group B acid and the control group (p<0.005).

Day 7

The mean and SD values of the compressive strength for group A, group B, and the control group after 7 days in MPa were (33.58±1.48), (34.64±2.21), and (56.29±4.84), respectively. One-way ANOVA showed a statistically significant difference between group A, group B, and the control group (p<0.001). The least compressive strength value was found in group A, followed by group B, and finally, the greatest compressive strength value was found in the control group. The Tukey post-hoc test revealed a statistically significant difference between the control group and each of the groups A and B, where (p<0.001). Also, there is no statistically significant difference between group A, and group B (p<0.739). **(Table 6) (Figure 9).** The study also showed an increase in the compressive strength across all the groups after 7 days

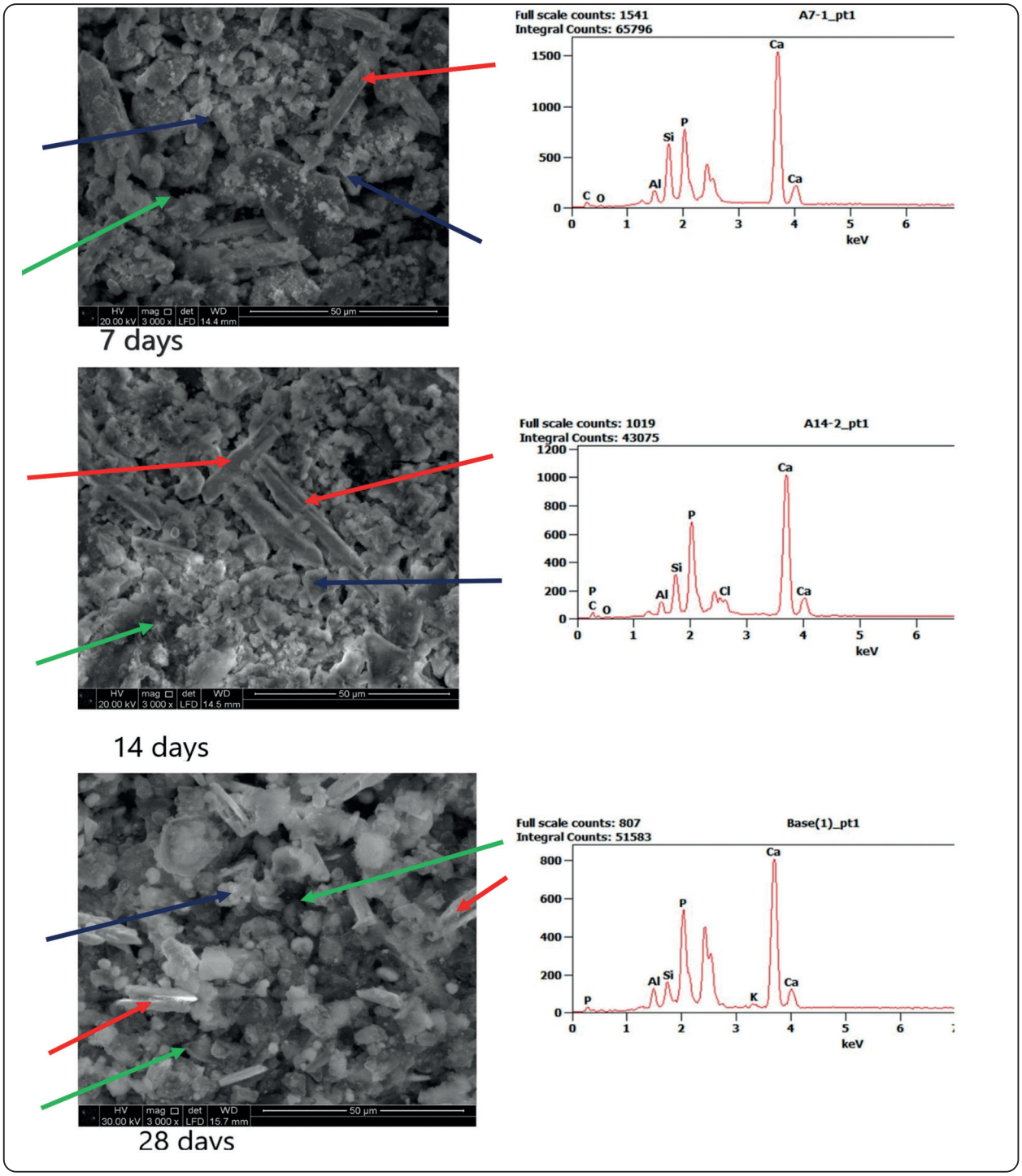

Fig. (5) SEM and EDX for Group A under 3000x magnification (Red arrows represent crystals, blue arrows represent spherical aggregates, green arrows represent porosity)

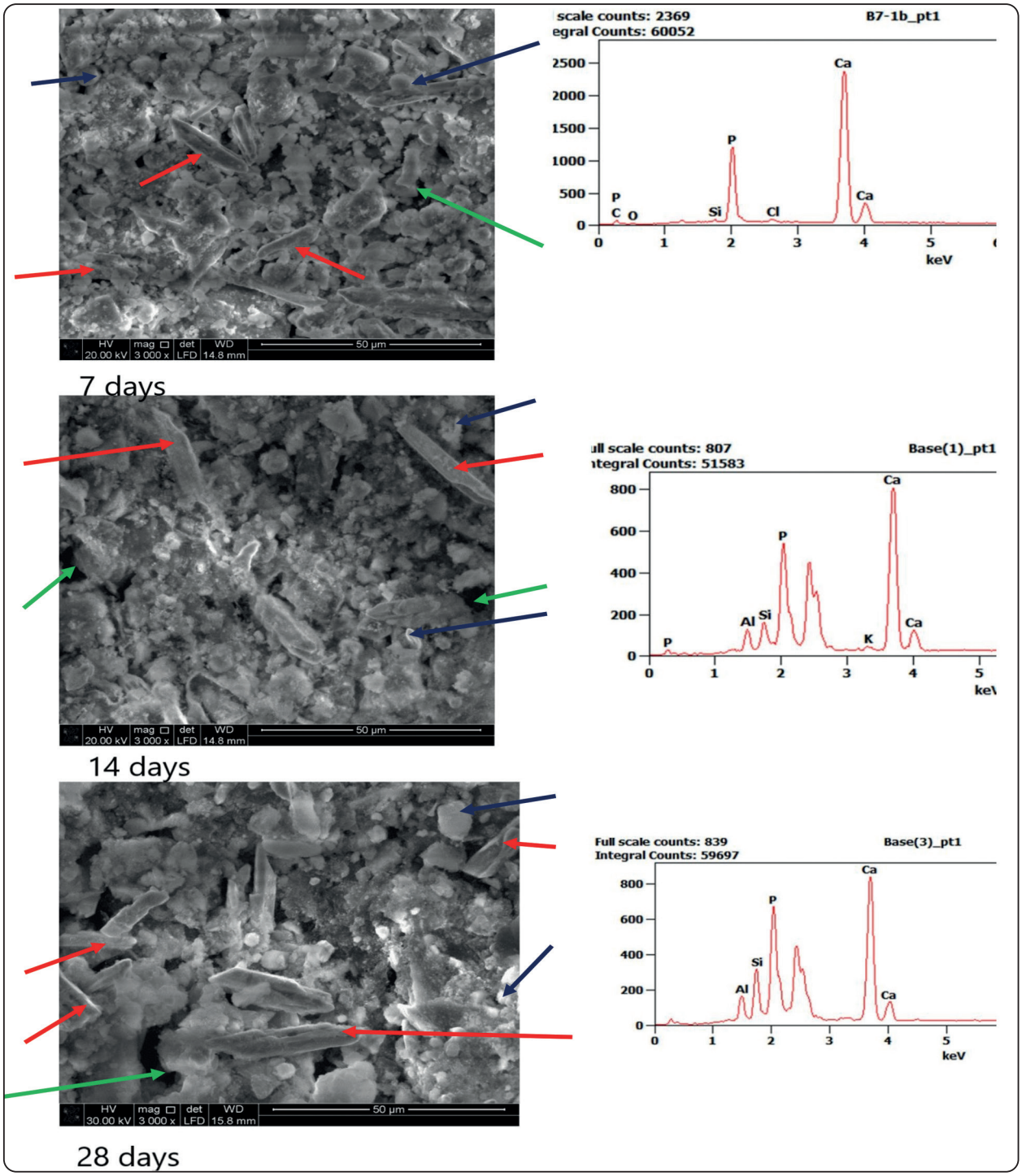

Fig. (6) SEM and EDX for Group B under 3000x magnification (Red arrows represent crystals, blue arrows represent spherical aggregates, green arrows represent porosity)

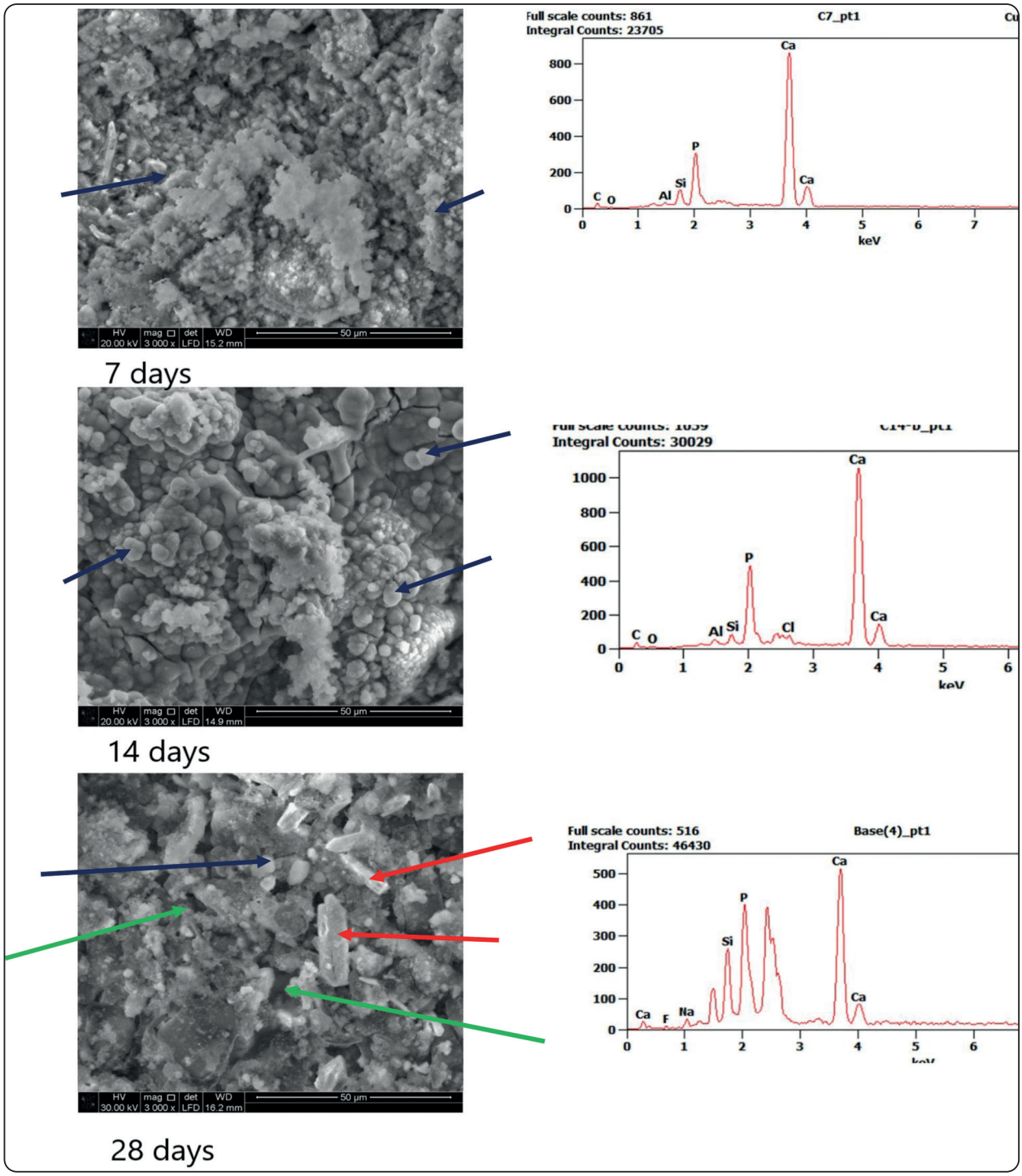

Fig. (7) SEM and EDX fot the Control group under 3000x magnification (Red arrows represent crystals, blue arrows represent spherical aggregates, green arrow represents porosity)

TABLE (2) The mean, standard deviation (SD) values of Cell viability (in %) using SRB cell assay

Variables	Viability %	
	Mean	SD
Group A	79.34	2.24
Group B	71.86	0.43
Control group	75.83	2.55

TABLE (3) The mean, standard deviation (SD) values of Calcium ion (in mg/L) release of different groups.

Variables	Calcium ion release (mg/L)					
	7 days		14 days		28 days	
	Mean	SD	Mean	SD	Mean	SD
Group A	11.17	0.81	14.25	0.70	15.99	0.59
Group B	10.92	1.23	16.67	1.27	24.27	0.61
Control group	7.72	0.67	10.73	2.46	13.85	0.27

TABLE (4) The mean, standard deviation (SD) values of the setting time (in minutes) of different groups.

Variables	Setting time (Minutes)				p-value
	Initial		Final		
	Mean	SD	Mean	SD	
Group A	5.95	1.22	21.01	1.10	**<0.001***
Group B	8.75	0.72	27.02	1.86	**<0.001***
Control	34.61	2.28	92.31	2.47	**<0.001***
p-value	**<0.001***		**<0.001***		

; significant (p<0.05)

TABLE (5) The mean, standard deviation (SD) values of compressive strength in MPa of different groups.

Variables	Compressive strength (MPa)				p-value
	Day 1		Day 7		
	Mean	SD	Mean	SD	
Group A	26.75	0.92	33.58	1.48	**<0.001***
Group B	31.32	1.38	34.64	2.21	**0.003***
Control	33.63	1.98	56.29	4.84	**<0.001***
p-value	**<0.001***		**<0.001***		

; significant (p<0.05)

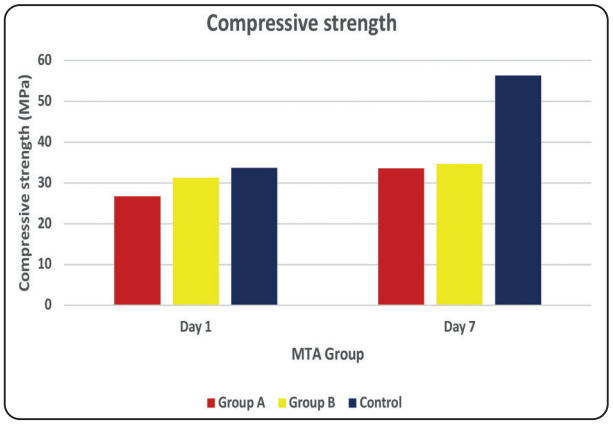

Fig. (8) Bar chart representing the setting time in minutes for different groups

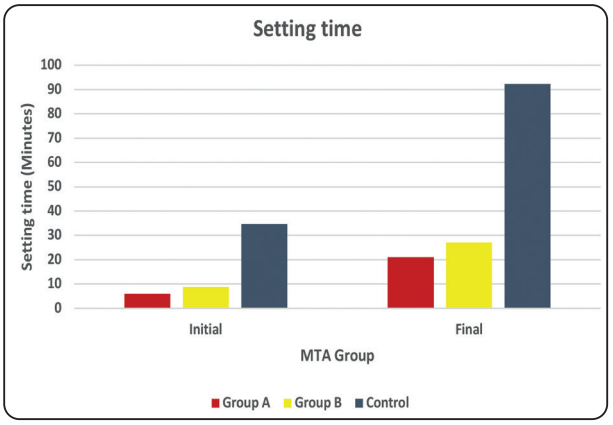

Fig. (9) Bar chart representing compressive strength in MPa for different groups

DISCUSSION

MTA is considered a promising and multipurpose material in dentistry. It is a gold standard material for direct and indirect pulp capping and in direct contact with periapical or periodontal tissues. MTA has several unique properties, including its ability to stimulate tissue regeneration, good pulp response, and good physical properties. **(Pushpalatha et al. 2022)** Despite the desirable qualities of MTA, long setting times and technique sensitivity are considered drawbacks. Considering MTA application in root canal treatment, compressive strength has great importance when placed in the furcation area and as pulp capping material; however, when placed as an apical plug where low applied force is needed, compressive strength is of lower importance**. (Kharouf et al. 2021)**

Phytic acid is an organic acid characterized by a distinctive structure of six phosphate groups bonded to an inositol ring. Phytic acid showed an acceleration in the setting of glass ionomer cement with minimal negative effects. Also, studies had shown a retarding effect on the setting of the calcium phosphate cements. **(Nassar et al. 2021)**

Recent studies have explored various modifications to MTA to enhance its biological, physical, and mechanical properties. The addition of different agents changed their physical and biological properties and may affect their clinical efficacy. **(Pushpalatha et al. 2022)** Accordingly, the present study aimed to study the effect of 2.5% and 5% aqueous solutions of phytic acid on the setting time and compressive strength of MTA.

Endodontic materials should exhibit high biocompatibility when they come in contact with living tissue, such as during perforation repair, retrograde filling, and pulp capping. Since the elution substance from endodontic cements gets access to periodontal tissue and can impact the healing process, its cytotoxicity must be investigated. **(Mathews, Hegde, and Thakur 2015)** In this study, a fresh cement mix of MTA mixed with phytic acid was used to prepare the extract to stimulate the clinical conditions. **(Knorr et al. 2021)**

The two concentrations of phytic acid used in the study were chosen after a pilot study to select the phytic acid concentration that showed acceptable cell viability according to ISO 10993-5. The SRB test is an effective, rapid, and inexpensive method that requires inexpensive reagents and simple equipment. This method depends on the binding ability of SRB dye to basic amino acids of the total cellular protein and doesn't rely on cellular function. **(Jaita et al. 2024)**

The results of the cell viability of MTA mixed with both concentrations of phytic acid showed that the cell viability was higher than 70%, which was considered cytocompatible according to the ISO 10993-5 directive. **(Knorr et al. 2021)** The cell viability of 2.5% phytic acid was higher than 5% phytic acid. This may be explained that increasing the concentration of phytic acid results in decreased cell viability due to the cytotoxic effect of the acidic solution. As phytic acid had a low pH**,** its use might cause cell death. This could be explained by the increase in the calcium ion release, which could affect the cells' survival. **(Youssef and Elsherief 2021**) These results came in agreement with **(Oh et al. 2016),** where the lower the concentration of the acid used, the higher the cell viability.

The test of calcium ion release showed that more calcium ions were released when 5% phytic acid was used than when 2.5% was used. This may be due to the higher concentration of phytic acid, which increased the acidity of the solution, leading to more solubility and ion release. These results were in accordance with **(Amin 2018),** which showed that the presence of acidic solution increased the calcium ion release, influencing the overall calcium release.

Bioactivity plays a key role in tissue regeneration and healing. The performance of calcium silicate is largely related to its bioactivity and its capacity to release calcium ions and form hydroxyapatite crystalline precipitate upon interaction with phosphate-rich physiological fluids. **(Talabani, Garib, and Masaeli 2020)** This bioactivity enhances cell proliferation and hard-tissue formation. **(Amin 2018)**

The results of the SEM revealed that all the tested materials in the study produced surface precipitates after incubation in simulated body fluid, and all the materials exhibited the formation of an apatite layer on their surfaces. These results were consistent with findings by some studies. **(Amin 2018) (Kamali et al. 2017)** It's worth mentioning that throughout all the different periods of incubation, the crystal morphology was continuously changing from the immature amorphous calcium phosphate phase to the apatite phase **(Figures 5, 6, and 7).** The apatite precipitate after 28 days was confirmed by EDX, where the Ca/P ratios were 1.45, 1.62, and 1.81, which denoted calcium-deficient hydroxyapatite, hydroxyapatite, and calcium-rich hydroxyapatite, respectively. The bioactivity of MTA mixed with a 5% aqueous solution of phytic acid showed more bioactivity than MTA with 2.5% phytic acid. This could be explained by the elevated concentration of phytic acid, which led to an increase in the calcium ion released, and also the presence of phosphate and silicate detected in EDX, as shown in (**Figures 5,6, and 7).** They would eventually react with ions from SBF, forming strong ionic interactions between phosphate and calcium ions, forming insoluble salts as hydroxyapatite on the cement surface. These findings were also observed by **(Kamali et al. 2017)**

MTA setting time is an important clinical property that affects the operative time and the ease of manipulation. A short setting time is required when MTA is placed against apical tissues and subjected to washout by blood, and it also enables the single-visit procedure. The cement setting is related to forming a solid network, which is associated with the hydration rate. **(Dianat et al. 2019) (Bogner et al. 2020)** Therefore, accelerating the hydration decreases the setting time. **(Abu-Nawareg and Zidan 2020)**

According to this study, the initial and the final setting time were found to be shorter in the two groups containing phytic acid compared to the control group. The reduction in the setting time was consistent with an earlier study by **(Uyanik et al. 2019),** which used a 1% aqueous solution of phytic acid. This was explained by the fact that phytic acid is extremely hydrophilic. It undergoes setting through a hydration reaction that is facilitated by its hydrophilic particles. Additionally, this may be due

to the high concentration of the phosphate group, which is negatively charged and has the affinity to bind to metal ions such as calcium ions present in MTA cement. As the number of phosphate groups increased, the binding to calcium ions increased, which may explain the short setting time of the experimental groups. Also, according to **(Sowjanya et al. 2020)**, phytic acid had a strong chelating ability, similar to chelating compounds such as EDTA.

Another explanation could be that the setting behaviour of the cements was influenced by the pH of the solution. When the cement was set in an acidic solution, the surface of the cement would simultaneously react with the acid. And this would lead to continuous leaching of ions, enhancing the permeability of the cement, and this would eventually accelerate the setting. Additionally, the setting may have been impacted by the local heat generated by the exothermic reaction between the cement and the acid. The elevated local temperature likely accelerated the reaction kinetics between the cement and the setting solution by enhancing ion mobility and increasing the solution's saturation level. **(Kamali et al. 2017)**

These findings were in contradiction to the study by **(Mahima et.al, 2021),** which showed no significant effect on setting time. These findings agreed with another study that explained that certain organic acids may not enhance the setting properties of MTA**.** These results could be explained by using a different acid than phytic acid. **(Möschner et al. 2009)**

However, since the 2.5% phytic acid group had a shorter setting time compared to the 5% phytic acid group, this could be explained by the fact that certain concentrations of accelerants exert a retarding effect; however, the lower concentrations tend to have an accelerating effect. This phenomenon, known as the poisoning effect, has been well documented in the context of gypsum-based materials. Gypsum accelerants such as borax and potassium sulphate are known to cause this phenomenon, as several salts are accelerators at low concentrations and retarders at higher concentrations. **(Chiayi S,et al 2022)**

High compressive strength of MTA is needed when MTA is used for pulp capping, furcation perforation, and perforation repair, as it needs additional force. Compressive strength measurement was done at two time periods, after 1 day and 7 days. After 7 days is the time when the material is commonly subjected to condensation forces of the restorative materials on top of it, as well as the mastication forces if two visits of endodontic treatment are used. Also, in the endodontic literature, 7 days is the studied time for evaluating the mechanical properties of CSCs. **(Amin 2018)** However, according to what was reported, the compressive strength was tested after placement in a moist environment containing distilled water to replicate the physiological conditions. **(Saghiri et al. 2013)** According to previous studies, most of the MTA accelerators have compromised the compressive strength of MTA.

According to the present study, the incorporation of phytic acid into MTA resulted in a reduction in the compressive strength of the MTA compared to distilled water, with a significant difference. One possible explanation for the reduction in the compressive strength was the intense effect of the acid-cement reactions, which reflected on the mechanical behaviour of MTA mixed with an acidic solution. This was also confirmed by the SEM micrographs **(Figures 5 and 6),** which showed a large number of pores in the microstructure due to the intense effect of the acid-cement reactions, which reflected on the mechanical behaviour of the MTA mixed with phytic acid. This was also confirmed by **(Kamali et al. 2017)**. According to **(Somaie et al. 2024),** there is an inverse relation between porosity and the mechanical properties, as

the presence of pores makes the material weaker. Therefore, without acidity, the corrosion would be less, and pore formation would be limited. So, the mechanical behaviour of MTA set in distilled water showed better behaviour due to the lack of the corrosive agent.

As explained during the study, 5% phytic acid showed higher compressive strength compared to 2.5% phytic acid. This could be explained by the fact that the longer the time of hydration, the more organized and rigid the crystalline microstructure, and therefore the better the mechanical properties. **(Bortoluzzi et al. 2019)** Therefore, 5% phytic acid, which had a longer hydration time, had higher compressive strength. The control group had the highest strength due to the absence of the corroding agent and the longer hydration rate. This was in contrast to other studies, which suggested that increasing the hydration rate improved the compressive strength of MTA**. (Saghiri et al. 2013), (Bortoluzzi et al. 2019)**

These results came in contrast to **(Hema et al.2024),** which showed that the addition of acid did not affect the compressive strength of the cement. The possible explanation could be that the other study used a different acid.

However, according to the literature, MTA compressive strength increased over time due to the slower hydration rate of dicalcium silicate. This finding is consistent with our study, where the three groups exhibited higher strength after 7 days compared to their one-day compressive strength. **(Saghiri et al. 2013)**

Based on the study results, the null hypothesis of this study has to be rejected, as the tested groups showed an effect on MTA setting time and compressive strength.

Under the conditions of our study, the two different concentrations of phytic acid can accelerate the setting; however, the compressive strength of MTA would be affected. Further in vivo studies are needed to evaluate the clinical applicability of using phytic acid with MTA.

CONCLUSIONS

The incorporation of phytic acid had a great influence on reducing the setting time of MTA; however, the compressive strength diminished. The shortest setting time of MTA was shown with the lowest concentration of phytic acid, with a decrease in the compressive strength of MTA.

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