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ORIGINAL RESEARCH

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Investigation of the effects of propolis and ozone addition on the physical and mechanical properties of MTA: an *in vitro* study

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Abstract

Background: Mineral trioxide aggregate (MTA), a calcium silicate–based cement introduced in 1993, is widely used in vital pulp therapy due to its excellent sealing ability, biocompatibility, and low cytotoxicity. This study added propolis (known for its antibacterial activity) and ozonated water to MTA to evaluate their effects on its physical and mechanical properties. **Methods**: Three experimental groups (n = 18 each) were created by mixing MTA with distilled water (control), propolis, or ozonated distilled water. Initial and final setting times were measured, Vickers microhardness was recorded at 1, 3, 7, and 28 days, and compressive strength was evaluated on day 28. Surface morphology and elemental composition were analyzed using Field Emission Scanning Electron Microscopy/Energy-Dispersive X-ray Spectroscopy (FE-SEM/EDS). Data were analyzed with repeated measures Analysis of Variance (ANOVA) or Friedman tests for intra-group comparisons and using one-way ANOVA or Kruskal-Wallis tests for inter-group comparisons ($p < 0.05$). **Results**: Propolis significantly prolonged both the initial (6 min) and final setting times (238.611 $\pm$ 4.804 min) compared with the other groups ($p < 0.001$), whereas ozonated water yielded values similar to those of the control. The compressive strength of the MTA + propolis group (30.749 $\pm$ 3.374 MPa) was significantly lower than that of the other groups ($p < 0.001$). Microhardness increased over time in all groups ($p < 0.001$), but the values in the propolis group remained consistently lower at all measurement points ($p < 0.001$). FE-SEM analyses revealed that crystalline formation was preserved in all groups, but samples with added propolis exhibited a film-like layer partially covering the surface. **Conclusions**: The addition of propolis adversely affected the physical and mechanical properties of MTA, whereas ozonated distilled water preserved its structural integrity, suggesting that ozonated water may be a safe alternative liquid for the clinical modification of MTA.

Keywords

Mineral trioxide aggregate; Antibacterial; Microhardness; Compressive strength

1. Introduction

Mineral trioxide aggregate (MTA) is a calcium silicate–based biomaterial widely used in vital pulp treatment due to its high biocompatibility, antibacterial activity, sealing capacity, and potential to induce hard-tissue formation. However, MTA has limitations in clinical application, including a long setting time and difficulty in manipulation [1–3]. Therefore, various modification studies in recent years have sought to enhance MTA's properties while preserving its biological advantages, aiming to reduce the setting time, enhance manipulability, and strengthen antimicrobial efficacy by modifying the powder or liquid composition [4–6]. To preserve the material's integrity and performance, it is essential to achieve these enhancements without adversely affecting its physical, chemical, and mechanical properties [4].

Propolis is a resinous substance produced by honey bees from various botanical sources. In addition to its low cytotoxicity, propolis has demonstrated antimicrobial activity against cariogenic bacteria, such as *Streptococcus mutans*, has been shown to enhance wound healing and has been used for root canal irrigation in endodontic treatment [7]. Its most important pharmacologically active components include phenolic compounds, aromatic substances, and flavonoids. The latter are well-known plant-derived compounds with antioxidant, antibacterial, antifungal, antiviral, and anti-inflammatory properties [8–10]. Research indicates that the flavonoids in propolis promote the formation of reparative dentin and inhibit inflammatory processes in the pulp. Consequently, propolis has been indicated for use in direct pulp capping and pulpotomy treatments [8, 11].

Ozone molecules are an unstable, energy-rich form of oxy-

gen, and ozone is a potent bactericidal agent that oxidizes microorganisms' cell walls and cytoplasmic membranes [12]. It can be applied in clinical dentistry in the form of ozone gas, ozonated water, and ozonated oil, of which the latter two can hold and subsequently release ozone [13]. In deep dentinal caries lesions, ozone therapy has been shown to reduce microbial load before final restoration [14], and laboratory studies have shown that ozone rapidly and significantly reduces the microbial load in carious dentin [15]. An *in vivo* study reported that ozone application effectively reduces Streptococcus mutans levels during cavity disinfection in primary teeth and provides a clinically applicable method of dentin surface disinfection prior to restorative treatment [16].

Pulp capping agents must possess optimum antimicrobial properties and biocompatibility, making it crucial to maximize MTA's antimicrobial properties while simultaneously ensuring optimal physico-mechanical properties [17]. Accordingly, propolis and ozonated water, known for their high biocompatibility, appear suitable as additives to enhance MTA's antimicrobial efficacy. Investigating the effects of these additives on MTA's physicochemical and mechanical properties is necessary to predict the material's clinical performance. This study examined the physical and mechanical properties of new formulations containing propolis and ozone intended to enhance MTA's antimicrobial activity and compared the findings with those of conventional MTA. The null hypothesis was that there would be no statistically significant difference in the physical and mechanical properties of MTA formulations containing propolis and ozone compared with conventional MTA.

2. Materials and methods

All experimental procedures of this *in vitro* study were conducted between August 2025 and October 2025.

2.1 Sample size calculation

Power analysis was performed using G*Power software (version 3.1.9.7; Heinrich Heine University, Düsseldorf, NRW, Germany). Based on the mean compressive strength values reported in 4th table of the reference study [4], a one-way analysis of variance (ANOVA) design was applied with an effect size of $f = 0.443$, a 95% confidence level ($1 - \alpha$), and a statistical power of 80% ($1 - \beta$). These parameters indicated a required sample size of 18 specimens per group.

2.2 Additives and experimental groups

This study used MTA Plus (Prevest DenPro Ltd., Jammu, India; LOT: PK24252377) as the experimental material. Propolis (crude propolis extract dissolved in 70% ethanol) and ozonated distilled water were added for their antibacterial properties. A 10% (w/v) propolis extract was prepared in 70% (v/v) ethanol according to the method described by Mejía *et al*. [18]. Ozonated water was prepared with an Ozonette Dent ozone generator (Ozonette Dent, Sedecal, Madrid, Spain) for 10 min at a flow rate of 30 L/h and a concentration of 60 μg/mL [15, 19]. The fresh solution was used within 5 min of preparation.

Three groups were established to evaluate the effects of the additives on the physico-mechanical properties of MTA:

Group 1 (control): MTA + distilled water;

Group 2: MTA + ozonated distilled water;

Group 3: MTA + propolis.

MTA powder was weighed using an electronic balance (Weightlab Instruments, WSA-224, Shanghai, China) with a precision of $\pm$0.0001 g, and the liquid components were measured with a micropipette (adjustable micropipette, Eppendorf Research Plus, Hamburg, HH, Germany) in the 1–1000 μL range. The powder/liquid ratio was maintained at 3:1 according to the manufacturer's instructions, and the mixing time did not exceed 1 min.

2.3 Sample preparation

Two different sets of samples were prepared in this study. For the setting-time evaluation, 18 specimens per group were fabricated using 10 $\times$ 2 mm stainless steel molds in accordance with ISO 6876:2025 (n = 54 in total) [20]. For the microhardness and compressive strength tests, 4 $\times$ 6 mm cylindrical specimens were prepared according to ISO 9917-1:2025 [21], with 18 samples per group (n = 54 in total). Altogether, 108 specimens were prepared, and all samples were stored in an incubator at 37 $\pm$ 1 °C and 100% humidity (NÜVE EN 055, NÜVE, Ankara, Türkiye).

2.4 Assessment of physical and mechanical parameters

The setting time was measured using a Vicat apparatus (manual Vicat needle apparatus, UTEST Materials Testing Equipment, Ankara, Türkiye) [4]. Microhardness was evaluated with a Vickers microhardness (VHN) device (HMV-G, Shimadzu, Kyoto, Japan) by applying 500 g load for 30 seconds on days 1, 3, 7, and 28; three repeat measurements were taken for each sample, and the average value was calculated [4]. The compressive strength test was performed on day 28 using a universal testing machine (Autograph AGS-X, Shimadzu Corporation, Kyoto, Japan) at a crosshead speed of 1 mm/min. For morphological examination and elemental analysis, the fractured samples obtained after compressive strength testing were gold-coated and imaged using FE-SEM (SU5000, Hitachi, Tokyo, Japan), and the elemental composition was evaluated using EDS (Oxford X-MaxN 80, Oxford Instruments, Abingdon, UK).

2.5 Statistical analysis

The data were analyzed using R statistical software (version 4.4.1). The normality of the data distribution was assessed using the Shapiro-Wilk test. For normally distributed variables, group comparisons were performed using one-way ANOVA, whereas the Kruskal-Wallis H test was used for non-normally distributed variables. For dependent data measured across multiple time points, the Friedman test was used for nonparametric variables and repeated-measures ANOVA for parametric variables. Because normality assumptions were not met for all time points, quantitative data are presented as mean $\pm$ standard deviation for normally distributed variables and

as median (minimum–maximum) for non-normally distributed variables. A p-value of < 0.05 was considered statistically significant.

3. Results

Statistically significant differences were observed between the groups for both the initial and final setting times ($p < 0.001$). The median initial setting time was significantly higher in the MTA + propolis group than in the other groups, while no difference was detected between the control and MTA + ozonated water groups.

Similarly, the final setting time was significantly increased by the addition of propolis, with the highest mean value observed in the MTA + propolis group. No significant difference was found between the control and ozonated water groups (Table 1).

As shown in Table 2, statistically significant differences were observed between the groups in compressive strength ($p < 0.001$), with mean strength values significantly lower in the MTA + propolis group. While no difference was observed between the control and MTA + ozonated water groups, the addition of propolis significantly reduced compressive strength compared with the other groups.

Tables 3 and 4 show statistically significant differences in microhardness values between the experimental groups and across the evaluated time points ($p < 0.001$), with microhardness values increasing significantly as time progressed in all groups (Fig. 1). While no significant difference was observed between the control and MTA + ozonated water groups at any measurement point, the MTA + propolis group's microhardness values were significantly lower at all time points ($p < 0.001$).

FE-SEM analysis revealed the characteristic surface morphology of MTA in all groups (Fig. 2a–c). In the control group, the surface exhibited a heterogeneous structure consisting of irregular particles. In samples mixed with ozonated water, the surface was more compact and residue-free. In samples with added propolis, a film-like structure partially covered the surface.

EDS analysis showed the atomic percentages (At%) of the elements found in the MTA samples (Fig. 3a–c). The primary elements in all three groups were oxygen (O), calcium (Ca), and carbon (C). The analyses also identified magnesium (Mg), silicon (Si), iron (Fe), aluminum (Al), bismuth (Bi), and gold (Au), the latter originating from the coating process.

4. Discussion

MTA offers superior biocompatibility and the capacity to support the regeneration of pulp and periradicular tissues and has a wide range of applications in dentistry [17]. However, its antibacterial activity is limited, and various approaches have been proposed to enhance it [22–24]. In this context, the use of natural and biocompatible agents has emerged as a significant research area for improving the material's clinical performance [7, 25, 26].

This study evaluated the effects of adding ozone and propolis—which are expected to increase antibacterial activity—on the physical, mechanical, and chemical properties of MTA. It was observed that MTA mixed with ozonated distilled water retained its physical and mechanical properties, whereas the addition of propolis prolonged the setting time and reduced mechanical strength. Based on the results, the hypothesis that adding ozonated water or propolis would not result in a significant difference in the physical and mechanical properties of MTA was rejected.

Ozone, owing to its strong oxidative capacity, exerts broad-spectrum antimicrobial activity by disrupting bacterial membranes and biofilm structures; it also supports tissue regeneration [27]. Makowiecki [28] reported that ozone application in vital pulp therapy contributes to pulp vitality by reducing bacterial contamination and that ozone may be safely used with calcium silicate-based materials; the article also emphasizes

TABLE 1. Comparison of initial (minute) and final setting time (minute) values according to groups.

	Control	MTA + ozonated water	MTA + propolis	Total	Test Statistic	p
Initial Setting Time	4 (4–5)^{a}	4 (4–5)^{a}	6 (5–6)^{b}	5 (4–6)	29.101	$<0.001^{x}$
Final Setting Time	134.333 ± 2.450^{b}	132.778 ± 2.798^{b}	238.611 ± 4.804^{a}	168.574 ± 50.111	5383.864	$<0.001^{y}$

^{x}Kruskal-Wallis H Test; ^{y}One-Way ANOVA; $^{a–b}$There is no difference between groups with the same letter. MTA: Mineral trioxide aggregate.

TABLE 2. Comparison of average compressive strength (MPa) values according to groups.

Groups	Mean ± SD	Median (min–max)	Test Statistic	p
Control	41.278 ± 6.729^{a}	41.305 (30.497–51.787)		
MTA + ozonated water	41.090 ± 4.754^{a}	41.458 (32.254–50.214)	36.071	$<0.001^{x}$
MTA + propolis	30.749 ± 3.374^{b}	31.848 (25.140–35.235)		

^{x}One-Way ANOVA; $^{a–b}$There is no difference between groups with the same letter. MTA: Mineral trioxide aggregate; SD: Standard deviation; min: minimum; max: maximum.

TABLE 3. Comparison of microhardness (Vickers Hardness, VHN) values according to groups.

	Control	MTA + ozonated water	MTA + propolis	Total	Test Statistic	*p*
T1	38.494 ± 1.653^{a}	37.284 ± 2.303^{a}	18.369 ± 1.878^{b}	31.382 ± 9.499	594.893	<0.001^{x}
T2	47.445 (41.2–50.0)^{a}	48.850 (42.8–51.2)^{a}	24.415 (19.5–29.9)^{b}	46.400 (19.5–51.2)	36.205	<0.001^{y}
T3	52.911 ± 3.154^{a}	51.953 ± 2.651^{a}	35.600 ± 3.083^{b}	46.822 ± 8.532	193.027	<0.001^{x}
T4	69.500 ± 3.461^{a}	68.200 ± 4.05^{a}	50.939 ± 4.892^{b}	62.880 ± 9.471	110.835	<0.001^{x}

^{x}One-Way ANOVA; ^{y}Kruskal-Wallis H Test; $^{a–b}$There is no difference between groups with the same letter. T1, T3, and T4 values are presented as mean ± standard deviation. T2 values are presented as median (minimum–maximum). MTA: Mineral trioxide aggregate.

TABLE 4. Comparison of microhardness (Vickers Hardness, VHN) values according to time within each group.

	Control	MTA + ozonated water	MTA + propolis
T1	38.494 ± 1.653^{a}	37.350 (32.7–40.2)^{a}	18.369 ± 1.878^{a}
T2	47.054 ± 2.212^{b}	48.850 (42.8–51.2)^{b}	24.724 ± 3.272^{b}
T3	52.911 ± 3.154^{c}	52.150 (48.1–57.4)^{c}	35.600 ± 3.083^{c}
T4	69.500 ± 3.461^{d}	68.400 (60.3–75.3)^{d}	50.939 ± 4.892^{d}
Test Statistic	539.048	52.867	394.127
p	<0.001^{z}	<0.001^{q}	<0.001^{z}

^{z}Repeated Measures ANOVA; ^{q}Friedman Test; $^{a–d}$There is no difference between time pointswith the same letter. MTA: Mineral trioxide aggregate.

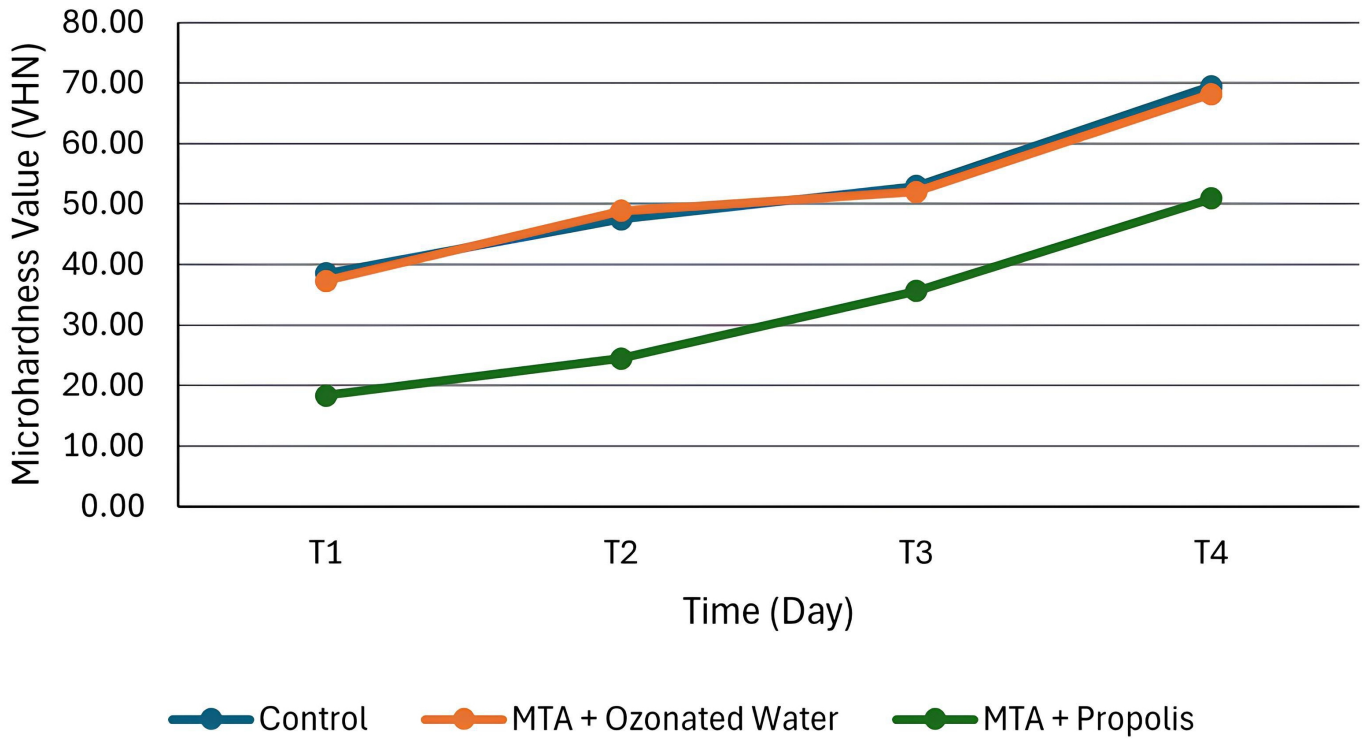

FIGURE 1. Distribution of microhardness values among groups across time intervals. Comparison of Vickers microhardness (VHN) measurements of the three MTA groups—control (MTA + distilled water), MTA + ozonated water, and MTA + propolis—evaluated at four different time points (1, 3, 7, and 28 days). MTA: Mineral trioxide aggregate.

that ozone exhibits antimicrobial activity and accelerates regenerative processes without altering the physical properties of dental hard tissues.

Propolis has been considered a potential biomaterial in regenerative endodontic applications due to its antimicrobial, anti-inflammatory, and regenerative biological properties. Studies in animal models have demonstrated that propolis increases root length and dentin thickness following revascularization in necrotic and immature permanent teeth and supports apical closure at a level comparable to MTA [29]. In addition, *in vitro* studies report that propolis preserves cell viability in human pulp cells, enhances mineralized tissue

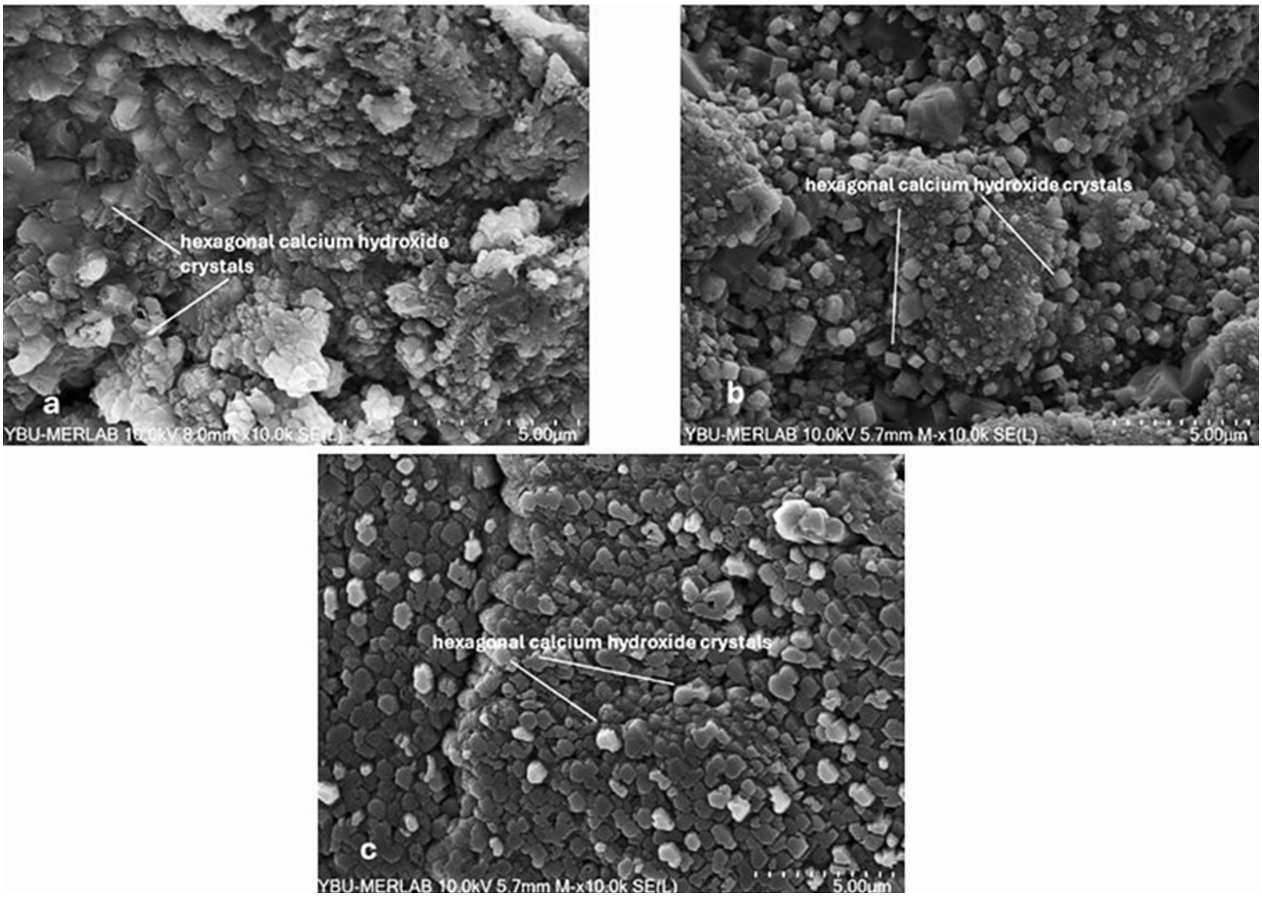

FIGURE 2. Surface morphology distribution based on FE-SEM analyses (10,000×). Representative FE-SEM images of MTA samples mixed with distilled water (control) (a), ozonated water (b), and propolis (c).

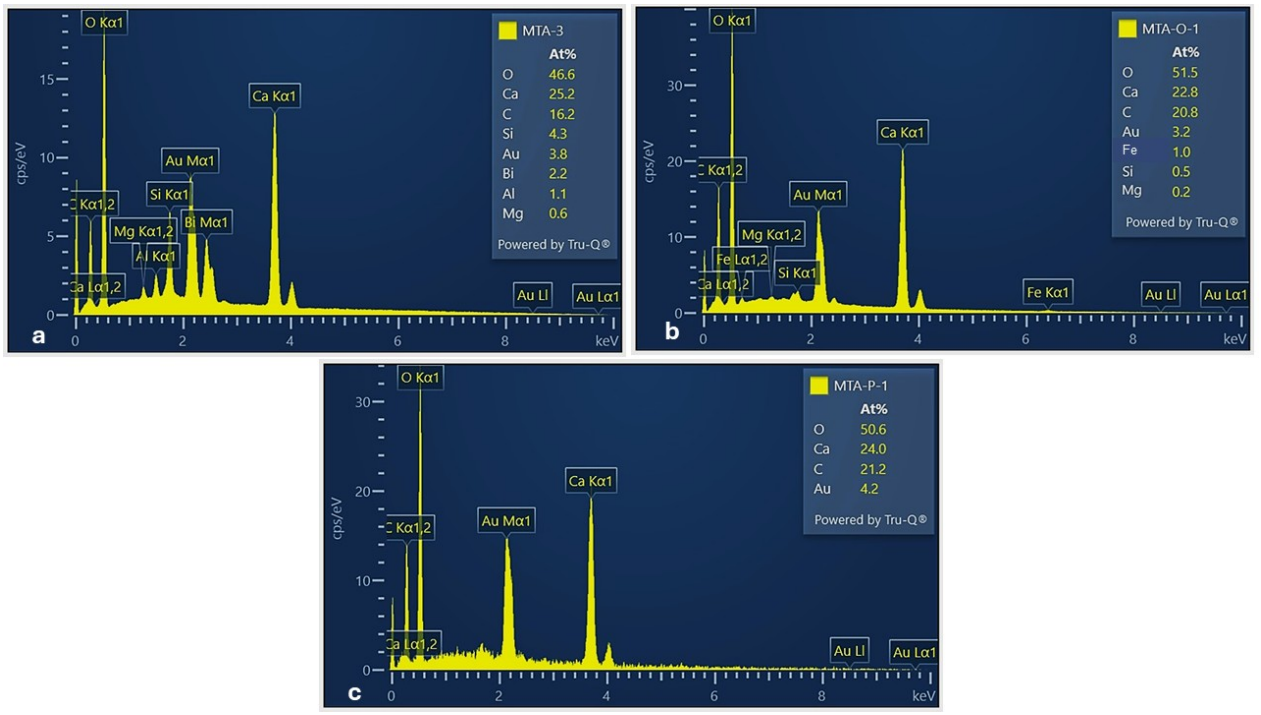

FIGURE 3. Representative EDS spectra of MTA groups. EDS spectra of samples mixed with distilled water (control) (a), ozonated distilled water (b), and propolis (c). O: oxygen; Ca: calcium; C: carbon; Si: silicon; Au: gold; Bi: bismuth; Al: aluminum; Mg: magnesium; Fe: iron; MTA: Mineral trioxide aggregate.

formation, and exhibits a pronounced anti-inflammatory effect [25]. Tiyapitsanupaisan *et al.* [26] showed that MTA mixed with propolis extract reduced matrix metalloproteinase-2 (MMP-2) expression in inflamed dental pulp cells, thereby supporting healing by limiting tissue destruction. Furthermore, incorporating propolis into MTA has been reported to increase early-stage cell proliferation and reduce cytotoxicity, thereby contributing to improved biocompatibility [7]. Ozone and propolis have been documented in the literature for their antibacterial activity and biocompatibility profiles, and their combined use with MTA is regarded as a clinically promising approach, but a significant knowledge gap remains in the extant literature regarding the effects of these combinations on MTA's physical and mechanical properties.

Previous studies [30, 31] have investigated combining MTA with various agents, such as sodium hypochlorite and chlorhexidine, to enhance its antibacterial properties. However, it has been reported that although these additives may increase antibacterial efficacy, they may also adversely affect MTA's biocompatibility and mechanical performance. These findings highlight the importance of evaluating not only the biological effects of additives but also their physical and mechanical consequences [17].

While the initial setting time of MTA is clinically essential for placement and manipulation of the material, the final setting time directly affects restorative procedures and sealing success [32]. In this study, the final setting time in the control group, in which MTA Plus was mixed with distilled water, was 134.333 ± 2.45 min, a result similar to the setting time of 128 ± 8 min that Camilleri *et al.* [33] reportedfor MTA Plus mixed with distilled water. Similarly, a study by Öztürk *et al.* [4] achieved a final setting time of 127.50 ± 4.82 min using MTA Plus mixed with distilled water. Moreover, the addition of ozonated water maintained the setting behavior of MTA without adversely affecting its hydration reactions, and the initial and final setting times were similar to those of the control group. In contrast, the addition of propolis significantly prolonged MTA's setting time.

In restorations subject to occlusal forces, it is clinically crucial that filling materials possess adequate compressive strength. MTA, used as a pulp-capping material, is also expected to provide sufficient resistance to the compressive forces generated by the restorative material placed on it [34]. This study recorded an average compressive strength of 41.27 ± 6.73 MPa for MTA Plus samples mixed with distilled water, which is within the same range as the MTA compressive strength results reported by Watts *et al.* [35] (57.4 ± 17.99 MPa) and Formosa *et al.* [36] (45.59 MPa), and is consistent with the literature.

In the present study, adding ozonated water did not significantly affect the compressive strength of MTA, yielding results similar to those of the control group. However, the average compressive strength in samples supplemented with propolis decreased to 30.74 ± 3.37 MPa. This can be explained by the addition of propolis, which may limit the duration of hydration. Indeed, previous studies have emphasized that mixing MTA with various additives may reduce the compressive strength of MTA due to the disruption of the crystallization process and particle interlocking [31, 37].

Microhardness is an essential indicator for evaluating the progress of hardening and the material's surface strength [38]. In this study, the microhardness of all groups increased gradually over 28 days, confirming that MTA gradually hardens completely within 4 weeks [39]. Formosa *et al.*'s [36] study using MTA Plus reports a microhardness value of 66.3 ± 6.25 VHN at 28 days. The VHN of 69.5 ± 3.46 obtained in our control group is consistent with this result and confirms that MTA Plus's hardening performance aligns with the literature. The addition of ozonated water preserved the hardening behavior without adversely affecting MTA hydration, and the microhardness values were comparable to those in the control group. In contrast, microhardness values were significantly lower in samples supplemented with propolis.

Propolis is a chemically complex natural product rich in polyphenols, flavonoids, wax-derived components, proteins, and other bioactive molecules [7]. Previous studies have demonstrated that exposure to organic- and protein-rich environments during MTA hydration can significantly alter crystal nucleation dynamics and microstructural development [40, 41]. Tingey *et al.* [41] reported that the surface of MTA set in the presence of fetal bovine serum, a protein-rich medium, exhibited a smoother, more globular morphology compared with the typical crystalline structures observed in water-set samples. Similarly, Nekoofar *et al.* [42] and Oloomi *et al.* [43] reported that protein-containing biological environments can affect MTA's microstructure by disrupting crystal formation, increasing porosity, and consequently reducing mechanical strength. Taken together, these findings indicate that organic- and protein-rich complex environments may adversely affect hydration reactions, crystal interlocking, and cement matrix densification. In this context, the rich organic composition of propolis may have similarly influenced hydration kinetics and microstructural organization, thereby contributing to the prolonged setting time, decreased compressive strength, and reduced microhardness values observed in the propolis-containing group in the present study.

In addition to these mechanisms, recent studies have confirmed the acidic properties of both non-commercial and commercial propolis extracts [44, 45]. Maghsoud Besharati *et al.* [45] reported that the pH values of propolis samples collected from different regions of Iran varied between 4.4 and 6.2 and that ethanolic propolis extracts created a similarly acidic environment.

The literature reports that MTA forms only cubic crystals in acidic media and that needle-like crystal structures disappear due to suppression of the hydration reaction [46]. Similarly, Lee *et al.* [38] note that MTA's hardness and hydration behavior decrease significantly inacidic conditions compared to physiological pH. Singh *et al.* [46] state that the compressive strength of MTA, which was 34.65 ± 11.98 MPa at pH 7.0, decreased to 30.14 ± 6.55 MPa at pH 5.0; furthermore, the low pH environment weakened MTA's crystallization structure, leading to significant deterioration in its mechanical properties. Similarly, Saghiri *et al.* [47] report that MTA's compressive strength decreased significantly as the environmental pH decreased. In addition, Watts *et al.* [35] reported that MTA's compressive strength decreased significantly when mixed with local anesthetic rather than sterile water and stored in an acidic

environment; however, this decrease was not observed in groups in which MTA was mixed with sterile water. In light of these findings, our study's observed adverse effects on the mechanical strength and setting time of MTA with added propolis may be attributable to its naturally acidic nature. Acidic additives lower the pH of the medium, thereby slowing hydration reactions, limiting crystal growth, and consequently weakening mechanical properties [35, 46].

In the FE-SEM analysis, calcium hydroxide (CH) crystals with the hexagonal morphology described in the literature were clearly observed in the MTA samples of the control group. This regular crystal structure is consistent with the typical hydration products of MTA and reflects the material's characteristic microstructure [38, 48]. In the other groups, the MTA-specific crystalline formation was also preserved. In samples containing propolis, a film-like organic layer was observed that partially covered the surface, a finding consistent with the smooth, globular surface morphology reported by Tingey *et al*. [41] for MTA set in the presence of fetal bovine serum. Considering that organic- and protein-rich environments can alter crystal formation dynamics, a similar mechanism may be associated with the surface alterations observed along with the prolonged setting time and reduced mechanical performance in the propolis-containing group.

EDS analyses showed that the elemental composition of MTA remained unchanged after mixing with distilled water, ozonated water, and propolis. This finding is consistent with the typical elemental distributions and calcium silicate hydrate (C-S-H)/CH products expected during MTA hydration [48, 49]. The detection of similar elements in all groups indicates that the additives did not alter the basic inorganic structure of MTA.

Due to its controlled laboratory conditions, this study is limited in its ability to fully reflect the biological conditions and long-term effects of the oral environment. Furthermore, the evaluations were limited to physical, mechanical, and surface properties; the biological cellular response and antibacterial activity were not directly assessed. Moreover, the use of a single MTA formulation and fixed propolis and ozone concentrations limited the ability to assess the potential effects of varied mixing ratios.

The results indicate that MTA maintains its physical integrity and hardening behavior when mixed with ozonated distilled water, whereas the addition of propolis may negatively affect the material's hydration. Therefore, while ozone addition may be a biocompatible modification method, propolis should be reevaluated using appropriate formulations and proportions.

5. Conclusions

This study's findings indicate thathe addition of ozone and propolis differently affected the physical and mechanical properties of MTA. The addition of ozonated water preserved the setting behavior and mechanical integrity of MTA without adversely affecting hydration. In contrast, the addition of propolis prolonged the setting time and reduced mechanical strength. Further studies evaluating different propolis concentrations and ozone application protocols together with antibacterial efficacy, will enable a more comprehensive understanding of the long-term biological and mechanical effects of these modifications.

AVAILABILITY OF DATA AND MATERIALS

The data supporting this study's findings are available on request from the corresponding author.

AUTHOR CONTRIBUTIONS

ZÖ—designed the research study and analyzed the data. ZÖ and BT—performed the research; wrote the manuscript. Both authors contributed to editorial changes in the manuscript. Both authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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