ORIGINAL RESEARCH

Comparative Evaluation of pH, Calcium Ion Release, and Setting Time of Premixed and Freshly Mixed Tricalcium Silicate-based Endodontic Materials: An *In Vitro* Study

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Abstract

Background and objective: During the last 200 years, there have been many changes in the way of performing endodontic treatment. The increased demand from patients for saving their teeth has led to the development of various innovative equipment and advances in the field of material sciences. Thus, the standard protocol of endodontic treatment has undergone several modifications. Bioceramic materials in endodontics can be considered a magnanimous entity with the ability to change the prognosis of many cases, especially those with poor prognosis.

Aim: The aim of this *in vitro* study was to compare and evaluate pH, calcium ion release, and setting time of premixed and freshly mixed tricalcium silicate-based endodontic materials.

Materials and methods: Polyethylene tubes measuring 10 mm in length × 1.0 mm in diameter were used. Group I (n = 15)—Polyethylene tubes filled with ProRoot MTA; group II (n = 15)—Polyethylene tubes filled with Biodentine; group III (n = 15)—Polyethylene tubes filled with NeoPutty MTA. Samples were placed in polypropylene flasks containing 10 mL of deionized water. At 6 hours, 12 hours, 24 hours, 7 days, and 1 month, the deionized water was measured for pH using a digital pH meter, and released calcium ions were measured by atomic absorption spectrophotometry. Ten specimens, each with an internal diameter of 10 mm and a thickness of 2 mm, were prepared for each material. A Vicat-type needle with a weight of 100 ± 0.5 gm and a flat end of 2.0 ± 0.1 mm in diameter was used to determine the initial setting time, and another Vicat-type needle with a weight of 456 ± 0.5 gm and a flat end of 1 ± 0.1 mm in diameter was used to determine the final setting time.

Results: The results of mean pH values of all three study groups showed that the overall mean pH value of ProRoot MTA (group I) was 10.99 ± 0.782, followed by group II, that is, Biodentine with 10.52 ± 1.108, and the least values were obtained from NeoPutty MTA (group III) with 9.77 ± 1.103, respectively (p-value > 0.001), which shows no statistically significant difference among the three groups. The mean values of calcium release observed in the study groups showed that the highest calcium ion release was shown by ProRoot MTA (group I) with 265.9 ± 136.7, followed by group II, that is, Biodentine with 108.8 ± 49.70, and the least values were obtained from NeoPutty MTA (group III) with 47.84 ± 24.05. All the experimental materials showed Ca^{2+} release at all time periods, with significantly higher calcium ion release observed in group I (ProRoot MTA). However, NeoPutty MTA showed the significantly lowest calcium ion release compared to the other two groups. This difference in the values of calcium ion release across all three groups was found to be statistically significant with a p-value = 0.005. Moreover, on evaluation of setting time, Biodentine showed faster initial and final setting times compared to the other groups.

Conclusion: The innovative endodontic cement, ProRoot MTA, exhibited an enhanced alkaline pH value and calcium ion release compared to Biodentine and NeoPutty MTA as bioceramic tricalcium silicate-based endodontic materials.

Keywords: Biodentine, Calcium silicate cement, NeoPutty, pH, ProRoot MTA.

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Introduction

Over the last 200 years, the techniques for performing endodontic treatment have evolved significantly. The growing demand from patients to preserve their teeth has led to the development of various innovative equipment and advances in the field of material sciences. Thus, the standard protocol of endodontic treatment has undergone several modifications. Bioceramic materials in endodontics can be regarded as a significant innovation that offers the ability to alter the prognosis of various cases, especially those with poor prognosis.[1]

Tricalcium silicate materials are commonly utilized in endodontics due to their excellent sealing properties, marginal adaptation, and bioactivity, making them suitable for various dental treatment modalities. Some of the commercially available tricalcium silicate endodontic materials include mineral trioxide aggregate (MTA Gray, MTA Angelus, Neo MTA Plus, NeoPutty MTA, etc.).[2]

Mineral trioxide aggregate (MTA) is an outstanding and superior biocompatible material introduced at Loma Linda University by Dr Torabinejad and his colleagues. It has numerous clinical applications, both nonsurgical and surgical, including temporary fillings, apexification, perforation repairs in roots or furcations, direct pulp capping, and root-end fillings. Despite its impressive

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clinical efficacy, several challenges have limited its use in certain cases, primarily its lengthy setting time and complex manipulation. To mitigate some of these drawbacks, various new calcium silicate-based materials have been created, with Biodentine being one of them.[2]

It is a recently introduced bioactive silicate cement in the dental market, marketed as a "dentin substitute." This biologically active compound penetrates open dentinal tubules, crystallizes, and interlocks with dentin, thereby enhancing its mechanical properties. Biodentine is marketed as a bioactive dentin substitute with active biosilicate technology. Similar to MTA, Biodentine belongs to the broader category of bioactive materials known as bioceramics, which consist primarily of tricalcium silicate cements.[3] Biodentine is a promising restorative material with improved handling characteristics. Even though Biodentine is a material of choice with excellent biocompatibility, it has certain limitations, including its cost and manipulation difficulties.[4] To overcome these drawbacks, NeoPutty MTA, a premixed syringe-type material, was recently introduced in the year 2020.

Since there is a paucity of studies on the physical and chemical properties of NeoPutty MTA, this study aimed to assess the calcium ion release, pH, and setting time of NeoPutty MTA in comparison to conventional MTA and Biodentine.

Aim

This *in vitro* study aimed to evaluate and compare calcium ion release, pH, and setting time of premixed and freshly mixed tricalcium silicate-based endodontic materials.

Materials and Methods

To Evaluate Calcium Ion Release and pH

Polyethylene N" tubes were cut into 45 equal-sized segments, each measuring 10 mm in length and 1.0 mm in diameter. The tubes were prewashed with 5% nitric acid to eliminate potential interference from phosphate ions and alkaline metals. The polyethylene-mounted tubes were then divided into the following groups:

Group I (n = 15)—ProRoot MTA (Fig. 1)

Group II (n = 15)—Biodentine (Fig. 2)

Group III (n = 15)—NeoPutty MTA (Fig. 3)

As per the manufacturer's instructions, the materials were freshly prepared and then transferred to the polyethylene tubes. After the tubes were completely filled, the materials were condensed with hand pluggers to eliminate any voids in the material. Subsequently, study samples were immersed in polypropylene flasks containing 10 mL of deionized water, which was verified to be free of calcium ions with a neutral pH of 6.8. The flasks were then sealed with lids, and the samples were stored in an incubator set to maintain a constant temperature of 37°C throughout the evaluation period. At 6 hours, 12 hours, 24 hours, 1 week, and 1 month intervals, the pH of the deionized water was assessed using a digital pH meter, and the released calcium ions were quantified using an atomic absorption spectrophotometer (Fig. 4).



Fig. 1: ProRoot MTA (group I)



Fig. 2: Biodentine (group II)



Fig. 3: NeoPutty MTA (group III)



Fig. 4: Calcium ion release measured by atomic absorption spectrophotometer

To Evaluate the Setting Time

Ten specimens with each material, each having an internal diameter of 10 mL and a thickness of 2 mL, were prepared. To determine the initial setting time, a Vicat-type needle weighing 100 ± 0.5 gm with a flat end of 2.0 ± 0.1 mm in diameter was used. For the final setting time, another Vicat-type needle weighing 456 ± 0.5 gm with a flat end of 1.0 ± 0.1 mm in diameter was employed (Fig. 5). The methodology, as described by Bortoluzzi et al., involved evaluations every 3 minutes during the first 30 minutes, every 5 minutes for the next 90 minutes, and every 15 minutes thereafter until the initial setting time was attained. The analysis proceeded at 15-minute intervals until the final setting time was established.

Results

In this study, calcium ion release and pH of each material were assessed at five time intervals: 6 hours, 12 hours, 24 hours, 7 days (1 week), and 30 days (1 month). The average pH values for each group are shown and compared in Table 1. It showed that the overall mean pH values exhibited by ProRoot MTA (group I) were 10.99 ± 0.782, followed by group I, that is, Biodentine, with 10.52 ± 1.108, and ultimately, the lowest values were observed in NeoPutty MTA (group III) with 9.77 ± 1.103. On comparison of pH values, no significant difference was revealed among the three groups. However, the pH values of ProRoot MTA were marginally higher compared to the other two groups, with a *p*-value > 0.001, which was statistically nonsignificant among the groups.

Additionally, Table 2 presents the average mean value of calcium ion release among the three study groups. It showed that ProRoot MTA (group I) exhibited the highest calcium ion release (265.9 ± 136.7), followed by group II, that is, Biodentine (108.8 ± 49.70), and the least values were obtained from NeoPutty MTA (47.84 ± 24.05). Although all the experimental materials demonstrated Ca^{2+} release throughout the time intervals, significant results were observed with group I (ProRoot MTA). However, NeoPutty MTA showed the significantly lowest calcium ion release compared to the other two groups. This variation in Ca^{2+} ion release noted among the three study groups showed statistically significant results.

When comparing calcium ion release and pH of each study group at different time intervals, it was found that ProRoot MTA exhibited a nearly constant alkaline pH along with significantly higher calcium ion release, which gradually decreased with increasing time intervals compared to the other two groups. The setting time of each group is illustrated in Table 3, which includes the initial and final setting times, respectively.

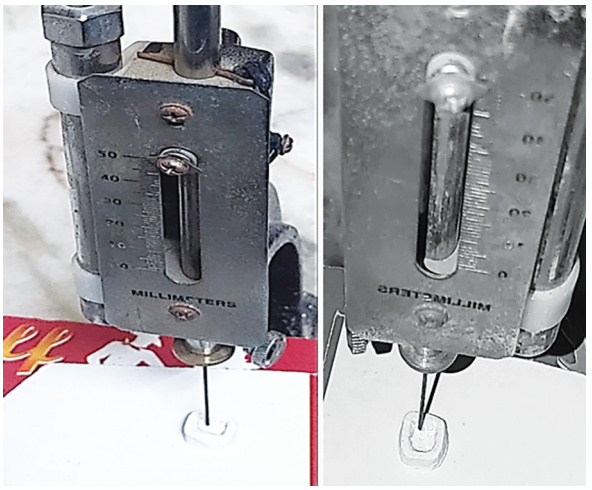

Fig. 5: Setting time evaluated using Vicat needle apparatus

Discussion

Several tricalcium silicate-based cements, differing slightly in composition and manufacturing processes, are available for endodontic applications. Noteworthy examples include ProRoot® white MTA, Medcem MTA®, and Biodentine™, all of which rely on tricalcium silicate as their primary ingredient. Furthermore, Mineral Trioxide Aggregate (MTA) has been the subject of extensive research, with a robust body of evidence supporting its clinical use in both endodontics and dental traumatology. Even though tricalcium silicate is the key component, the properties of these cements may vary due to differences in radiopacifiers, manufacturing techniques, material purity, and the hydration products formed.[5]

Tricalcium silicate cements are predominantly composed of calcium-based compounds and produce calcium hydroxide both

Table 3: Initial and final setting time of all three groups

Group	*Initial setting*	*Final setting*
ProRoot	35 ± 5.24	175 ± 1.48
Biodentine	12 ± 3.48	45 ± 1.28
NeoPutty	170 ± 1.58	292 ± 5.55

Table 1: Comparison of test parameter pH among three groups using ANOVA

Test parameter	*Groups*	*Mean ± SD*	*F*	*p-value*
pH	ProRoot	10.99 ± 0.782	1.868	0.197
	Biodentine	10.52 ± 1.108		
	NeoPutty	9.77 ± 1.103		

Table 2: Comparison of test parameter calcium ion release among three groups using ANOVA

Test parameter	*Groups*	*Mean ± SD*	*F*	*p-value*
Calcium ion release	ProRoot	265.9 ± 136.7	8.729	0.005*
	Biodentine	108.8 ± 49.70		
	NeoPutty	47.84 ± 24.05		

*$p < 0.05$

during and after the setting process. Tricalcium silicate cements are widely used in endodontics and dental traumatology for procedures including pulp capping, pulpotomy, perforation repair, apexification, root resorption treatment, root-end filling, and root canal obturation.[1] In addition to these well-established applications, probative research is exploring the potential of recently introduced commercial calcium silicate biomaterials for regenerative endodontic therapy and in the reinforcement of weakened endodontically treated teeth, especially when combined with posts, temporary restoration of deep caries, and dentin replacement. These indications stem from the ability of tricalcium silicate cements to form calcium hydroxide during hydration, which dissociates into calcium and hydroxide ions. The calcium ions then activate signaling pathways crucial for mineralization, while hydroxide ions generate an alkaline environment that enhances antibacterial and anti-inflammatory effects.[5,6]

In our study, pH was also taken as a primary test parameter because it detects the material's acidic or alkaline level. The high alkalinity of tricalcium silicate cements can induce dentinal collagen denaturation, facilitating the infiltration of Ca^{2+} ions and the exchange of minerals. This process helps form a "mineral infiltration zone" at the interface between the dentin and Biodentine. Several studies have demonstrated that calcium hydroxide is the end product of the hydration of these tricalcium-based materials, with calcium ions being the predominant ions released during the setting process. Since calcium ions were found to be the predominant ions during the hydration process of endodontic materials, calcium ion release was taken as a second test parameter in our study. Furthermore, it is also an important parameter that determines an ideal endodontic material.[7]

The ion release process from calcium silicate-based materials facilitates crystal deposition on their surface, initiating the precipitation of hydroxyapatite (HA). HA, being rich in calcium ions, offers excellent biocompatibility, reduces tissue toxicity and foreign body reactions, supports osteoid induction, and produces a favorable osteogenic effect. The ability to release calcium ions is considered a crucial factor for successful endodontic treatment, as it facilitates mineralization of dentin and aids in the formation of a dentin bridge.[7,8]

Thirdly, setting time was considered a key parameter, being a necessary physical characteristic for endodontic materials. Setting time is one of the vital characteristic properties of endodontic materials, as it can influence the material's strength. Dental materials should have properties that influence their strength. The setting process begins once an endodontic material is blended and manipulated with the appropriate liquid. This process involves two key intervals based on time: initial and final setting time, which mark the stages of the setting process. The initial setting time is the period during which the material reaches a semi-hardened consistency, while final setting time is the duration from mixing until the material is completely hardened and can be safely removed from the mold. The setting time can be estimated by penetration testing, such as the Vicat needle apparatus, which is found to be an effective technique for determining the setting time of endodontic materials. This method was evaluated in the present study and is also a standard method for evaluating the setting time, readily available in our country, and used to determine the setting time in accordance with American National Standards Institute/American Dental Association (ANSI/ADA) specifications, with molds of dimensions 10 × 2 mm used.[7]

In the present study, polyethylene tubes were used in place of extracted teeth due to the difficulty in standardizing the foramen opening. To ensure consistent test conditions, pH and calcium release were measured in deionized water free of ions. And so, test specimens immersed in deionized water, with readings taken at 6 hours, 12 hours, 24 hours, 7 days, and 30 days.[9,10]

Subsequently, this study reported that ProRoot MTA maintained a higher alkaline pH even after one month. Since an alkaline environment plays a key role in facilitating the healing of pulp tissue and promoting remineralization, ProRoot MTA showed a high alkaline pH level, which was maintained throughout the study period of 30 days/1 month. An alkaline environment acts as a favorable factor for the differentiation of fibroblasts into odontoblasts, thereby facilitating the formation of a calcified dentin bridge. It is believed that a strong alkaline pH can extend the setting time, thereby enhancing a long-lasting antibacterial effect, thus eliminating any residual microbes that may survive on the dentinal wall.[6]

The high pH of MTA, which peaks around 6 hours, is attributed to the calcium oxide present in its composition. The capability of MTA to release calcium hydroxide may have clinical relevance, as it has the potential to stimulate mineralization and promote the formation of reparative dentin.[11]

Furthermore, MTA released significantly higher levels of calcium ions (Ca^{2+}) but drastically decreased after 28 days. Nonetheless, the release of Ca^{2+} from all materials declined over time. MTA exhibited higher levels of calcium release than Biodentine for up to 7 days, likely due to MTA's higher content of calcium-based constituents. The lower release of calcium ions from Biodentine can be attributed to its rapid hydration reaction, which leads to accelerated setting of the material. This fast-setting process results in reduced solubility and consequently, lower release of Ca^{2+} ions. After 28 days, Ca^{2+} release from Biodentine increased and exceeded that of MTA. This is likely due to the inclusion of calcium chloride in the Biodentine liquid, while MTA uses deionized water in its liquid, which may account for the differences in calcium ion release between the two materials. MTA quickly releases Ca^{2+} and generates an alkaline environment, which stimulates crystallization and nucleation of B-type hydroxycarbonate apatite.[11,12]

The antibacterial and antifungal properties of endodontic bioceramic materials are mainly attributed to their elevated pH. This increased alkalinity of these materials prevents microbial growth, aiding in the disinfection of dentin. Hiremath et al. assessed the antimicrobial properties of bioceramic materials, finding that both MTA and Biodentine showed notable antimicrobial activity against *Enterococcus faecalis*, while MTA Plus was particularly effective as an antifungal agent against *Candida albicans*.[13]

The release of Ca^{2+} ions and subsequent increase in pH, closely linked to the duration of setting and solubility of a material, supports tissue repair while also promoting apatite formation.[14,15] The incremental release of ions from ProRoot MTA may be linked to its higher calcium concentration in the composition of the endodontic cement. However, the sudden decrease in calcium ion release after 14 days across all cements may result from a decline in calcium ion dissolution over time. MTA released calcium ions at a faster rate than Biodentine, primarily because Biodentine has

lower solubility. This reduced solubility is linked to the hydrosoluble polymer in the Biodentine liquid, which functions as a water-reducing agent.[16]

Compared with ProRoot MTA and Biodentine, NeoPutty MTA showed less calcium ion release and lower alkaline pH values. This may be attributed to its premixed type, which likely results in a decrease in calcium ion dissolution over a longer period compared to freshly mixed cements. Consequently, the hypothesis that the three materials differ in their ability to form apatite crystals and release Ca^{2+} was validated, which could be based on the calcium hydroxide concentration and variations in their alkaline pH levels. Furthermore, it was demonstrated that calcium carbonate actively contributes to the hydration reaction by providing nucleation sites, which accelerates the setting kinetics. The absence of these elements in NeoPutty MTA may explain the observed decrease in calcium ion release over time, as well as the lower leaching compared to both Biodentine and MTA. This could be attributed to the lack of dicalcium silicate in NeoPutty MTA, a component associated with slower hydration kinetics, which is linked to delayed hydration reactions and low solubility.[17]

Upon evaluating the setting time, this study concluded that Biodentine has a final setting time ranging from 12 to 45 minutes, while ProRoot MTA showed a setting time of approximately 2 hours and 45 minutes, and NeoPutty MTA exhibited a longer setting time of about 4 hours. This indicates that NeoPutty MTA has a significantly longer setting time, which may be considered one of its major drawbacks. The faster setting time of Biodentine observed in this study may be attributed to the inclusion of a setting accelerator, which facilitates rapid setting and improves both its strength and handling characteristics. On the other hand, the prolonged setting time of MTA, as noted by Torabinejad et al., could elevate the risk of partial loss of material and interface disruption during the finishing phase. Therefore, Biodentine presents a substantial improvement over MTA, particularly in terms of setting time. Additionally, the extended setting time of NeoPutty MTA may be due to the absence of a setting accelerator in its composition, which was identified as a drawback of this material.[17,18]

Conclusion

The clinical application of bioceramic materials has surged in recent years due to their wide-ranging benefits and broad applicability in restorative dentistry and endodontics. While ProRoot MTA, as an innovative endodontic cement, possesses beneficial characteristics such as enhanced Ca^{2+} ion release and an alkaline pH, it also presents certain limitations, including challenging manipulation, higher cost, and a slow setting time. These drawbacks have prompted researchers to explore alternatives that offer improved handling properties and cost-effectiveness, without compromising the material's biological and clinical efficacy.

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