

Chemical Constitution, Morphological Characteristics, and Biological Properties of ProRoot Mineral Trioxide Aggregate and Ortho Mineral Trioxide Aggregate

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Purpose: This study sought to compare the elemental constitution, morphological characteristics, particle size distribution, biocompatibility, and mineralization potential of Ortho MTA (OMTA) and ProRoot MTA (PMTA).

Materials and Methods: OMTA and PMTA were compared using energy-dispersive spectrometry, particle size analysis, and scanning electron microscopy. The biocompatibility and mineralization-related gene expression (osteonectin and osteopontin) of both MTAs were also compared using methylthiazol tetrazolium assay and reverse transcription-polymerization chain reaction analysis, respectively. The results were analyzed by Kruskal-Wallis test with Bonferroni correction. P-value of <0.05 was considered significant.

Result: The morphology of OMTA powders was similar to that of PMTA. The constituent elements of both MTAs were calcium, silicon, and aluminum. The mean particle sizes of OMTA and PMTA were 4.60 and 3.34 μ m, respectively. Both MTAs had equally favorable in vitro biocompatibility and affected the messenger RNA expression of osteonectin and osteopontin.

Conclusion: Within the limitations of this study, OMTA could be a promising biomaterial in clinical endodontics.

Key Words: Biocompatibility; Energy-dispersive spectrometry; Mineralization-related gene expression; Ortho MTA; Particle size analyzer; Scanning electron microscopy

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Introduction

Mineral trioxide aggregate (ProRoot MTA; Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) has been used successfully as root-end filling material^{1,2}, perforation repair material³, and pulp-capping material⁴. The good clinical performance of mineral trioxide aggregate (MTA) is attributed to its good sealing ability^{5,6}, biocompatibility^{1,7}, and capacity to promote mineralized tissue formation⁸. These properties of MTA stem from its many desirable characteristics, including its elemental constitution⁹ and particle size distribution¹⁰.

The constituent compounds of MTA are important in determining its characteristics since these could affect the setting time, compressive strength, and biological properties of MTA¹¹. The particle size of MTA is also crucial, considering the fact that it could affect the handling properties, surface area, and biological activity of the material¹⁰. One previous study reported that the smaller particle size of Portland cement increased the setting reaction rate and improved its early age strength¹². Investigations evaluating the biocompatibility of MTA and its ability to promote the formation of mineralized tissue are also essential because the

material could be in direct contact with pulpal or periradicular tissues.

Many bioactive ceramic types of cement that could be used as alternatives to ProRoot MTA have been developed, and these have been introduced in the dental market worldwide¹³⁻¹⁵. One such cement, Ortho MTA (BioMTA Ltd., Seoul, Korea), was recently developed in Korea, and it satisfies the International Standardization Organization regulation concerning arsenic and lead contents¹⁶. The suggested use of this material is largely the same as that for ProRoot MTA.

Considering the fact that the heavy metal content of Ortho MTA was reported to be lower than that of ProRoot MTA¹⁷, this material could be considered one possible alternative to ProRoot MTA. Because Ortho MTA is a newly developed material, however, no published studies are available as to its chemical and morphological characteristics, cytotoxicity, or mineralization potential. Therefore, this present study sought to compare the chemical constitution, biocompatibility, particle size distribution, and ability to promote mineralized tissue formation of Ortho MTA and ProRoot MTA.

Table 1. Components of the mineral trioxide aggregate (MTA) materials tested in this study

Commercial brand of MTA	Component
ProRoot MTA (tooth colored formula)	Tricalcium silicate (CaO) ₃ •SiO ₂
	Dicalcium silicate (CaO) ₂ •SiO ₂
	Tricalcium aluminate (CaO) ₃ •Al ₂ O ₃
	Tetracalcium aluminoferrite (CaO) ₄ •Al ₂ O ₃ •Fe ₂ O ₃
	Gypsum, CaSO ₄ •2H ₂ O
	Free calcium oxide, CaO
	Bismuth oxide, Bi ₂ O ₃
	Tricalcium silicate (CaO) ₃ •SiO ₂
	Dicalcium silicate (CaO) ₂ •SiO ₂
Ortho MTA	Tricalcium aluminate (CaO) ₃ •Al ₂ O ₃
	Tetracalcium aluminoferrite (CaO) ₄ •Al ₂ O ₃ •Fe ₂ O ₃
	Free calcium oxide, CaO
	Bismuth oxide, Bi ₂ O ₃
	Bismuth oxide, Bi ₂ O ₃

The ProRoot MTA was from Dentsply Tulsa Dental Specialties, and the Ortho MTA was from BioMTA.

Materials and Methods

In this study, Ortho MTA (Lot #O 1005 T 30 A 1, BioMTA) and ProRoot MTA (Lot #10003598A, Dentsply Tulsa Dental Specialties) were used. The main components of ProRoot MTA are tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, gypsum, and bismuth oxide. The main components of Ortho MTA are tricalcium silicate and dicalcium silicate. Note, however, that Ortho MTA is free of gypsum. The components of ProRoot MTA and Ortho MTA are shown in Table 1.

1. Morphological and Chemical Composition Analyses

The morphological examination of Ortho MTA powder compared with ProRoot MTA powder was carried out using scanning electron microscope (SEM, Model X5000 S4700; Hitachi, Schaumburg, IL, USA; $\times 500$), and elemental analysis was performed with an energy-dispersive spectrometer (EDS) attached to SEM. A thin layer of each powder was dispersed over a polymethyl methacrylate slab mounted on an aluminum stub. Each specimen was coated with carbon (K250; Emitech, Ashford, UK) for electrical conductivity.

2. Particle Size Analysis

Particle size analysis of Ortho MTA and ProRoot MTA was also done. Each sample was dispersed in distilled water. The particle-to-dispersant ratio was 0.0023% by volume, and a light-scattering method was used to detect the particle size of the two samples. A Mastersizer 2,000 particle size analyzer

(Malvern Instruments Ltd., Worcestershire, UK) was used for this analysis. The mean particle size and the particle size distribution of each sample were investigated using this method.

3. Cell Culture and Cytotoxicity Test Using Methylthiazol Tetrazolium Assay

MG-63 (CRL-1427) human osteosarcoma cells obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol) fetal bovine serum (Gibco, Grand Island, NY, USA) and 1% (wt/vol) antibiotics/antimycotics (100 U of penicillin, 100 μ g of streptomycin, and 0.25 μ g of amphotericin B; Gibco) at 37°C in a humidified 5% (vol/vol) CO₂ atmosphere. One gram of ProRoot MTA or Ortho MTA was mixed according to the manufacturer's instructions. After mixing, the materials were placed in sterilized molding rings with inner diameter of 5 mm and thickness of 1 mm. Any excess material was removed using sterile blades, and the materials were left to set for 3 hours at room temperature in 97% humidity. One milliliter of medium containing 5×10^4 MG-63 cells was seeded into each well of the 24-well plates. The cells were cultured with a culture plate insert with MTA specimen on the top surface of the culture plate insert. Intermediate restorative material (IRM, Dentsply Tulsa Dental Specialties) and empty tubes were used as positive and negative controls. After 1, 3, and 7 days of incubation, cell viability was assessed using an methylthiazol tetrazolium (MTT) (3-[4,5-dimethylthiazol-2yl]-2,5-diphenyl-2H-tetrazolium bromide) assay kit. The cells were

Table 2. Reverse transcriptase-polymerase chain reaction primers sequence

Gene	Sequence (5'-3')	Size (bp)
Osteonectin	Forward: AGA AGC TGC GGG TGA AGA A	405
	Reverse: TGC CAG TGT ACA GGG AAG ATG	
Osteopontin	Forward: CCA AGT AAG TCC AAC GAA AG	347
	Reverse: GGT GAT GTC CTC GTC TGT A	

incubated with 5.7 mol/L of MTT solution for 4 hours in a tissue culture incubator. Afterward, 200 μ l dimethyl sulfoxide solution was added to the cell culture wells, with the plates shaken for 10 minutes at room temperature to dissolve the precipitated formazan crystals. The solution was centrifuged for 10 minutes, and the optical density of the supernatant was measured at 540 nm using an

enzyme-linked immunosorbent assay plate reader (PowerWave; BioTek Instruments, Winooski, VT, USA).

4. RNA Isolation and Reverse Transcription-polymerase Chain Reaction

After 1, 3, and 7 days of culture, the total RNA of the incubated cells was extracted using Trizol

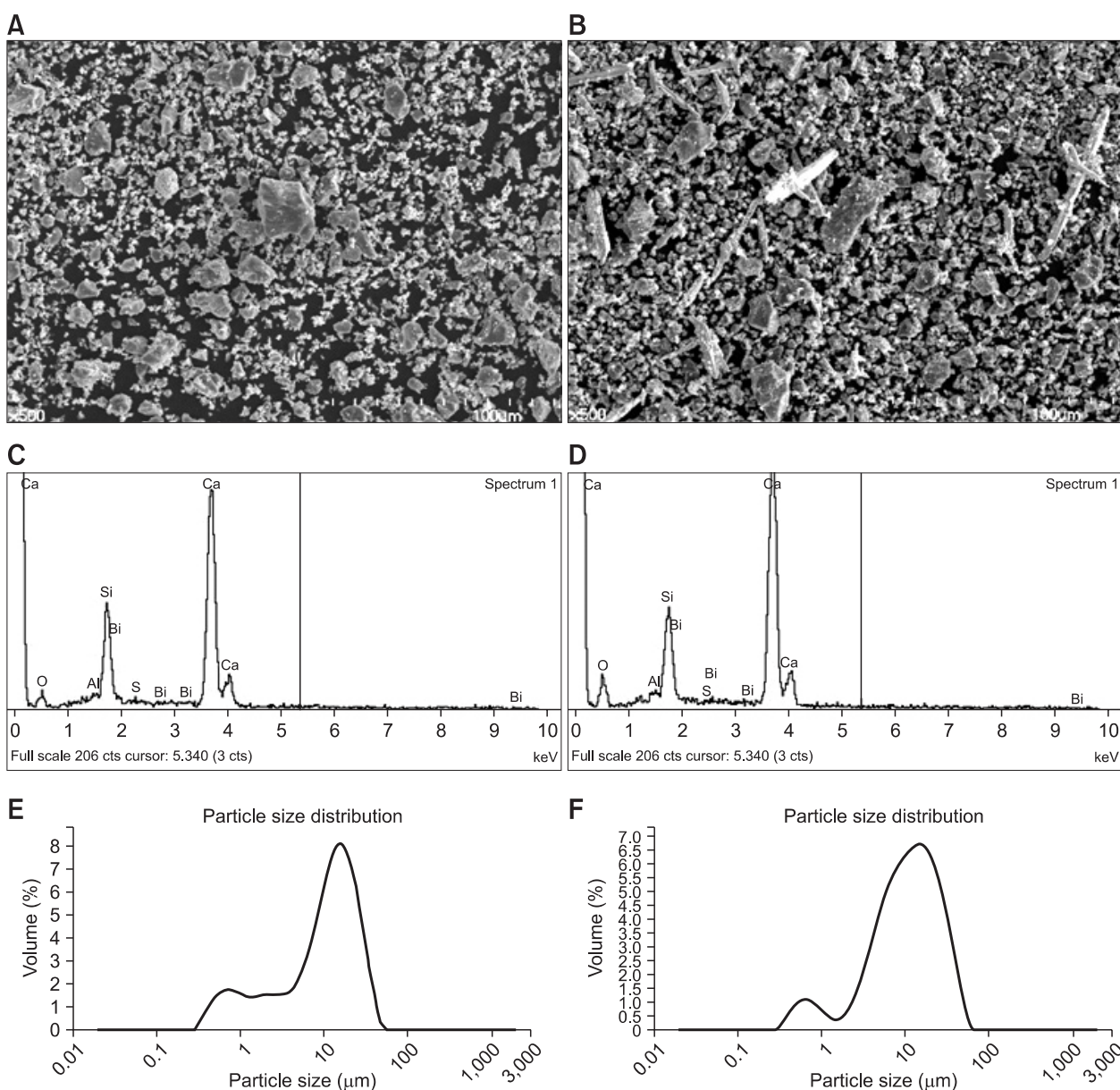


Fig. 1. Morphological appearance of ProRoot MTA (A) and Ortho MTA (B) by scanning electron microscopy (x5,000). The energy-dispersive spectrometry analysis showed similar compositional elements in the ProRoot (C) and Ortho (D) MTAs. The particle size distributions of ProRoot MTA (E) and Ortho MTA (F) showed little difference. MTA: mineral trioxide aggregate.

reagent (Life Technologies, Gaithersburg, MD, USA) according to the manufacturer's recommendations. Reverse transcription (RT) of RNA was performed using an AccuPower RT Premix (Bioneer, Daejeon, Korea), which was also utilized for amplifying the RT-generated DNA. Primer sequences and their major functions for osteonectin (ON) and osteopontin (OPN) are detailed in Table 2. The polymerase chain reaction (PCR) products were resolved on a 1.5% agarose gel and stained with ethidium bromide. A gel image was recorded and analyzed using Gel Doc (Bio-Rad, Hercules, CA, USA) and were normalized to the housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase, as a template.

5. Statistical Analysis

Statistical analyses of the MTT assay and RT-PCR data were carried out using the Kruskal-Wallis test

with Bonferroni correction (SPSS Statistics version 17.0; SPSS Inc., Chicago, IL, USA). The confidence interval was 95%, and a P-value of <0.05 was considered significant.

Result

1. Scanning Electron Microscope and Energy-dispersive Spectrometer Analyses

The SEM examination revealed that both ProRoot MTA and Ortho MTA have relatively homogeneous powder, and that both contain some larger particles (Fig. 1A, 1B). The EDS analysis showed that both MTAs were composed mainly of elements such as calcium, silicon, and aluminum (Fig. 1C, 1D).

2. Particle Size Analysis

The mean particle size of ProRoot MTA was 3.34 μm , whereas that of Ortho MTA was 4.60

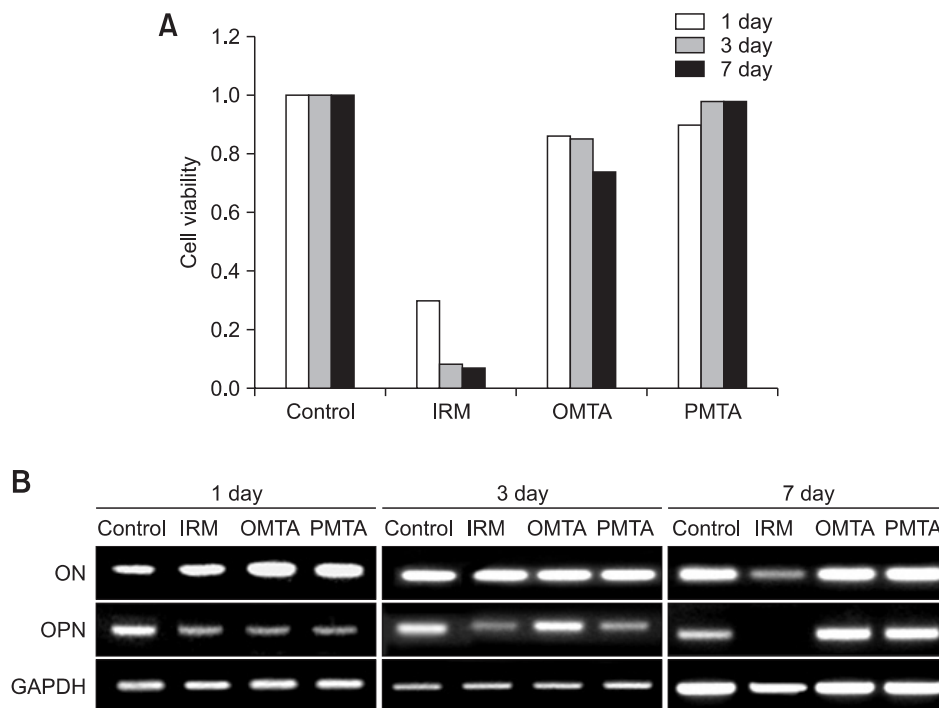


Fig. 2. Results of the methylthiazol tetrazolium assay and reverse transcription-polymerase chain reaction analysis (A). Cell viability was assessed after incubating MG-63 cells for 1, 3, and 7 days with intermediate restorative material (IRM), ProRoot MTA (PMTA) or Ortho MTA (OMTA). (B) The messenger RNA expression of osteonectin (ON), osteopontin (OPN), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was assessed after incubating MG-63 cells with IRM, PMTA or OMTA for 1, 3, and 7 days (x200). MTA: mineral trioxide aggregate.

µm. Mastersizer graphs revealed that the particle sizes of both MTA cements were in the range of approximately 0.3~50 µm (Fig. 1E, 1F).

3. Cytotoxicity Test Using MTT Assay

Both MTAs showed statistically higher cell viability values than IRM at all incubation times (Fig. 2A). Compared with the control, the percentage of viable cells was 90% for ProRoot MTA and 86% for Ortho MTA after 1 day of incubation. After 3 days of incubation, the percentage of viable cells was 98% for ProRoot MTA and 85% for Ortho MTA; after 7 days of incubation, cell viability was 98% for ProRoot MTA and 74% for Ortho MTA (Fig. 2A). ProRoot MTA and Ortho MTA showed no statistically significant difference in cell viability at any incubation time ($P>0.05$).

4. RNA Isolation and Reverse Transcription-Polymerase Chain Reaction

After 1 day of incubation, the messenger RNA (mRNA) expression of ON significantly increased in both MTA groups ($P<0.05$), whereas the mRNA expression of OPN were decreased in both MTAs ($P<0.05$) compared with the control groups. After 3 days of incubation, the mRNA expression of OPN increased in Ortho MTA compared with those in the control and IRM groups ($P<0.05$). After 7 days of incubation, the mRNA expression of OPN increased for ProRoot MTA compared with that of the control groups ($P<0.05$). However, no significant differences in the mRNA expression of ON were observed between the groups ($P>0.05$).

Discussion

This study compared the chemical constitution, particle size, biocompatibility, and mineralization potential of Ortho MTA and ProRoot MTA. With regard to the morphological characteristics of the two MTAs, particles in the ProRoot MTA appeared relatively homogeneous, with some

large particles. These results are consistent with the findings of previous studies^{9,18}. The morphological characteristics of Ortho MTA were similar to those of ProRoot MTA.

The particle size of Portland cement is known to affect its handling characteristics¹⁹. To our knowledge, however, the only study that investigated the particle size of ProRoot MTA is that by Komabayashi and Spångberg¹⁰. The authors reported that the mean particle size of ProRoot MTA was 2.96 µm. The mean particle size of ProRoot MTA in our study was 3.34 µm, which was in the same range as that of Komabayashi and Spångberg¹⁰. Additionally, we found that the mean particle size of Ortho MTA (4.60 µm) was larger than that of ProRoot MTA. The effect of such size difference on the physical properties of Ortho MTA needs to be determined in future studies.

Several studies have investigated the chemical composition of ProRoot MTA²⁰⁻²². The main elements of ProRoot MTA have been reported to be calcium, silicon, and aluminum^{9,23}, and this was confirmed in the present study. The main elements detected in the Ortho MTA were almost similar to those of ProRoot MTA. Note, however, that the previous study of Chang et al.¹⁶ revealed that ProRoot MTA contained a little amount of arsenic (1.16 ppm), whereas Ortho MTA was free of arsenic. Furthermore, they reported that Ortho MTA contained significantly lower levels of heavy metals (cadmium, chromium, copper, iron, manganese, and nickel) than ProRoot MTA¹⁷. The effect of these differences in heavy metal content on the biochemical properties of MTA need further investigation.

Previous studies have shown that ProRoot MTA is biocompatible with and nontoxic to pulpal and periradicular tissues^{9,24,25}. The present study showed that the cytotoxicity of both MTAs was significantly less than that of IRM and found no significant difference between the two MTAs. These results suggest that the biocompatibility of Ortho MTA is

comparable with that of ProRoot MTA. Most of the previous studies have evaluated the cytotoxicity of MTA for a relatively short duration^{9,25}. Considering the fact that ProRoot MTA maintains high pH and high calcium ion release for up to 7 days²⁶, an evaluation of the cellular response to MTA for 1 week may be more clinically relevant. In addition, in the present study, the MTA samples themselves were cultured with the MG-63 cells^{25,27}, whereas other studies have used MTA eluates^{28,29}. Using the samples of MTA themselves instead of MTA eluates more closely simulates the clinical situations wherein pulpal or periradicular tissues are in direct contact with MTA.

Among the various genes known to be related to the formation of mineralized tissue, we investigated the mRNA expression of ON and OPN because the two have been reported to be involved in bone initiation, mineralization, and remodeling^{30,31}. The present study showed that ProRoot MTA increased the mRNA expression of ON after 1 day of incubation and that of OPN after 7 days of incubation compared to the control group. Generally, there was no significant difference in the mRNA expression of ON and OPN between the two MTAs. Reichert et al.³⁰ reported that ON, a major non-collagenous matrix protein in bone and dentin, plays a role in the initiation of mineralization. On the other hand, Zhang et al.³¹ stated that OPN takes part in the formation of mineralized tissue. The present results suggest that both MTA cements have the potential to promote mineralization.

Conclusion

The two MTA cements have similar chemical compositions and morphological characteristics. The particle size of ProRoot MTA was slightly smaller than that of Ortho MTA. Both MTA showed good biocompatibility and upregulation of mineralization-related gene expression.

Considering the previous report on Ortho MTA having lower heavy metal content than ProRoot MTA¹⁷, these results suggest that Ortho MTA could be a useful endodontic biomaterial. Note, however, that this was an *in vitro* study, and further *in vivo* investigation of the tissue compatibility of Ortho MTA and its dentin/cementum-formation potential is necessary to evaluate the usefulness of Ortho MTA as a possible alternative to ProRoot MTA.

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