

Ion Release, Porosity, Solubility, and Bioactivity of MTA Plus Tricalcium Silicate

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Abstract

Introduction: The aim of this study was to evaluate MTA Plus (Prevest Denpro Limited, Jammu, India, for Avalon Biomed Inc) material's properties, namely calcium release, the pH change, solubility, water sorption, porosity, surface morphology, and apatite-forming ability after immersion in simulated body fluid. **Methods:** Two tricalcium silicate powders (MTA Plus and ProRoot MTA; Dentsply Tulsa Specialties, Tulsa, OK) and Dycal (Dentsply Caulk, Milford, DE) were tested. After incubation at 37°C and 99% relative humidity, calcium and hydroxyl ion release were tested up to 28 days in deionized water at 37°C. Water absorption, interconnected pores, apparent porosity, and solubility were measured after 24 hours of immersion in deionized water at 37°C. The morphologic and elemental analysis of the materials' surfaces were examined using an environmental scanning electron microscope/energy dispersive x-ray analysis after storage at 37°C for 1–28 days in simulated body fluid using the ISO 23317 method. **Results:** All 3 materials created an alkaline pH within 3 hours, which continued for 28 days. MTA Plus had a higher ion release than ProRoot MTA and Dycal; the use of the MTA Plus gel enhanced the initial calcium release and the increase of the pH. Both MTA materials were more porous, water soluble, and water sorptive than Dycal and more bioactive. After aging in simulated body fluid, MTA Plus material caused precipitation of an apparent calcium phosphate layer. **Conclusions:** MTA Plus showed improved reactivity and prolonged capability to release calcium and increase the local pH to alkaline values in comparison with ProRoot MTA. These pronounced ion-releasing properties are interlinked with its noticeable porosity, water sorption, and solubility and with the formation of calcium phosphorus minerals. The finer calcium silicate powder may explain the higher ion release, water sorption, porosity, and solubility of MTA Plus compared with ProRoot MTA. For clinicians, MTA Plus represents a lower-cost bioactive tricalcium

silicate material with interesting chemical-physical properties that could be a convenient alternative to the conventional calcium silicate mineral trioxide aggregate–like cements. (*J Endod* 2014;40:1632–1637)

Key Words

Calcium phosphate formation, ion releasing, mineral trioxide aggregate, MTA Plus, porosity, ProRoot MTA, root-end filling materials, solubility, tricalcium silicate, water sorption

Mineral trioxide aggregate (MTA) products are hydrophilic powders composed of tri- and dicalcium silicates. These hydraulic materials set with the addition of water to form calcium silicate hydrate gel (1–3). Portland cement is composed of primarily tri- and dicalcium silicates and was used in dentistry in 1878 (4); it was patented in 1995 when it was combined with bismuth oxide powder (3, 5).

Gray (5) and tooth-colored (also known as white) (6) ProRoot MTA (Dentsply Tulsa Specialties, Tulsa, OK) has revolutionized root-end therapy and pulp capping procedures in relation to the property to set in moist/bloody environments or sites contaminated by biological fluids (2, 7), the ability to stimulate the formation of hydroxyapatite (1, 2), and the generation of a flux of calcium and hydroxyl ions through dentin as recently shown by Gandolfi (8).

MTA Plus (Prevest Denpro Limited, Jammu, India, for Avalon Biomed Inc) is a finer powder, lower-cost product that has a composition similar to tooth-colored ProRoot MTA (9) and is proposed for treating dental pulp (pulp capping, cavity lining, and pulpotomies) and root canals (root-end filling, perforation repair, root resorption, apexification, and obturation in pulpectomy).

The MTA Plus kit includes 2 mixing liquids: a proprietary salt-free polymer gel and water. MTA Plus is indicated as a root canal sealer as well as a root-end filling material and a pulp capping cement. By using the gel and varying the powder to gel ratio, different setting times and physical-rheologic properties can be obtained. The gel has been formulated to confer washout resistance, whereas its fine powder particle size improves handling and placement (10). Recent studies showed the possibility to perform retreatment of teeth filled with MTA cements (10, 11) although it has been suggested to avoid filling procedures using MTA-like cements to completely obturate the root canal because the collagen and flexural strength of the dentin can be negatively affected (12, 13).

The aim of the present study was to compare the properties, namely calcium release, pH, solubility, water sorption, porosity, surface morphology, and apatite-forming ability after immersion in simulated body fluid (SBF), of 3 materials.

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Materials and Methods

Materials

MTA Plus (lot 41001), tooth-colored ProRoot MTA (lot 09003850), and Dycal (Dentsply Caulk, Milford, DE; lot 81007) were tested. ProRoot MTA and Dycal represented the comparison commercial materials for root-end filling and pulp capping procedures, respectively.

MTA Plus and ProRoot MTA were mixed for 30 seconds on a glass slab with their liquids using a liquid to powder ratio of 0.31 for ProRoot MTA and 0.37 for MTA Plus. MTA Plus powder was also mixed with its gel at 0.33. To prepare Dycal, base and catalyst pastes were mixed equally. Dycal (14) contains calcium hydroxide ($\text{Ca}[\text{OH}]_2$) in a salicylate base. Freshly mixed pastes were compacted into polyvinyl chloride molds (8 ± 0.1 mm diameter \times 1.6 ± 0.1 mm), and the excess was removed. The exposed upper surface area of each sample was 50.24 ± 0.01 mm².

Ion Release

The molds were placed on the bottom of cylindrical polystyrene containers (3-cm high with a 4-cm diameter) with 10 mL deionized water, sealed, and stored at 37°C. The water was collected and renewed after 3 hours and 1, 7, 14, and 28 days. Calcium ion and pH measurements were performed of the water until the measurement stabilized. Deionized water was the negative control.

pH. The pH measurements were performed on the collected water with a pH probe (Sen Tix Sur WTW, Weilheim, Germany) connected to a multiparameter meter (inoLab 750; WTW, Weilheim, Germany).

Calcium Release. After the pH measurement, the solution was supplemented with 200 μL (2%) ionic strength adjuster (4 mol/L KCl, WTW). Measurements were made using a calcium ion selective probe (Eutech Instruments Pte Ltd, Singapore). The cumulative release of calcium was calculated.

Porosity, Solubility, and Water Sorption

Another set of disks was set at 37°C and 99% relative humidity for 70% of their setting time (55 minutes for MTA Plus and 250 minutes for ProRoot MTA). The specimens were unmolded, dried with filter paper, and weighed (initial mass, D_i). Each disk was immersed vertically in 20 mL distilled water at 37°C. After 24 hours, the mass while suspended in water (S) was determined. Excess water was removed, and the saturated mass (M) was recorded. Samples were dried at 37°C to a stable weight (dry mass [D_f]). Each weight measurement was repeated 3 times to the nearest 0.001 g using an analytical balance (Bel Engineering series M, Monza, Italy).

The exterior volume ($V [M - S]$), the volume of open pores ($V_{\text{OP}} [M - D]$), the volume of impervious portion ($V_{\text{IP}} [V_{\text{IP}} = D_f - S]$), and the apparent porosity ($P [(M - D_i)/V] \times 100$) were calculated following Archimedes' principle (American Society for Testing and Materials C373 [2006]). The water sorption ($A [(M - D_f)/D_f] \times 100$) and the solubility ($S [S = ((D_i - D_f)/D_f) \times 100]$) were calculated (15, 16).

Statistical Analysis

Two-way repeated measures analysis of variance statistical analysis was performed with the Student-Newman Keuls test ($P < .05$).

Bioactivity Test

The ISO 23317 (Implants for surgery—*In vitro* evaluation for apatite-forming ability of implant materials) method was used to evaluate layers precipitated on the materials soaked in SBF. Molds filled

with freshly prepared cement pastes (set for about 10 minutes) were placed vertically in 20 mL Hank's Balanced Salt Solution (HBSS; Cambrex Bio Science Verviers Sprl, Verviers, Belgium [cat. n.10-527]) for use as SBF. Each sample's exposed surface was 100.48 ± 0.01 mm² (including upper and lower surfaces), and the surface/volume ratio was $100.48/20 = 5.024$ (15). HBSS was replaced weekly.

Freshly prepared samples (approximately 5 minutes after mixing) and samples aged in HBSS for 1, 7, or 28 days were examined "wet" using an environmental scanning electron microscope (ESEM; Zeiss EVO 50; Carl Zeiss, Oberkochen, Germany) connected to a secondary electron detector for energy dispersive x-ray analysis (EDX; Oxford INCA 350 EDS, Abingdon, UK) (2). EDX provided qualitative and semi-quantitative measurements of atomic calcium and phosphorous to calculate the superficial calcium to phosphorus (Ca/P) atomic ratios (17). The formation of calcium carbonate (CaCO_3) was presumed when the Ca/P ratio exceeded 1.67.

Results

Ion Release

Table 1, sections A and B, contain the pH values and calcium ion values (mean \pm standard deviation, $n = 10$) for all soaking times. All 3 materials created an elevated pH (alkaline) after 3 hours of soaking, with the highest value for MTA Plus mixed with gel (12.0). Over 28 days, all materials gradually decreased in their rate of release of hydroxyl ions, and the pH diminished. After 28 days, the pH was the highest for Dycal solutions (9.8) and the lowest for ProRoot MTA eluate (7.1).

The calcium ion release after 3 hours was highest from MTA Plus, mixed with water (43 ppm) or gel (119 ppm), and lowest for Dycal (25 ppm) and ProRoot MTA (24 ppm). The calcium ion release diminished for all materials over 28 days. At 28 days, MTA Plus with gel had a release of 19 ppm compared with ProRoot MTA, Dycal, and MTA Plus with water (16, 16, and 8 ppm, respectively).

Porosity, Solubility, and Water Sorption

The apparent porosity varied from 9% for Dycal to 40% for MTA Plus mixed with water or gel (39%) as shown in Table 1, section C. The water sorption and the solubility followed the same trends. Water sorption was only 5% for Dycal and highest for MTA Plus with gel (26.5%). The solubility was lowest for Dycal and highest for MTA Plus powder with water (18.5%).

Bioactivity Tests

The ESEM/EDX results for freshly mixed MTA Plus with gel (Fig. 1) revealed compounds of calcium, silicon, bismuth, aluminum, and carbon on the surface. From this, we concluded that MTA Plus contains calcium silicates, bismuth oxide, a minor amount of calcium aluminate, and a carbon-containing polymer. After 7 days, a precipitate had formed on MTA Plus that contained compounds of calcium, sodium, magnesium, phosphorous, and chlorine with a Ca/P ratio of 4.45. The carbon peak was no longer observed, indicating the precipitate formed from HBSS completely covering the surface. The atomic percentage of calcium and phosphorous increased for the 28-day sample, whereas all other elemental peaks diminished, except for sodium and chlorine, which is consistent with the precipitation of NaCl and a calcium phosphate phase. The Ca/P ratio was 2.47, which is higher than that of hydroxyapatite.

The freshly mixed and unexposed MTA Plus mixed with water (Fig. 2) had major EDX peaks for calcium, silicon, and bismuth and lesser peaks for sodium, sulfur, aluminum, and potassium, whose origins can be attributed to minor constituents of cement but may have been obscured in the gel sample. When soaked in HBSS, the bismuth

TABLE 1. (A) Calcium Release in the Soaking Water; (B) pH of the Soaking Water; and (C) Porosity, Water Sorption, and Solubility of the Materials

	3 hours	1 day	3 days	7 days	14 days	28 days
(A) pH of soaking water						
MTA Plus	11.7 ± 0.30 ^{Aa}	11.54 ± 0.56 ^{Aa}	10.0 ± 0.89 ^{Ba}	8.64 ± 0.50 ^{Ca}	8.67 ± 0.34 ^{Ca}	8.29 ± 0.51 ^{Ca}
MTA Plus gel	12.05 ± 0.22 ^{Aa}	12.47 ± 0.30 ^{Ab}	10.68 ± 1.4 ^{Bb}	9.77 ± 1.00 ^{Cb}	8.55 ± 0.65 ^{Da}	7.99 ± 0.23 ^{Da}
ProRoot MTA	11.07 ± 0.47 ^{Ab}	10.58 ± 0.55 ^{Ac}	9.16 ± 0.45 ^{Bc}	8.87 ± 0.12 ^{Ba}	7.75 ± 0.39 ^{cb}	7.12 ± 0.07 ^{cb}
Dycal	10.29 ± 0.48 ^{Ac}	10.49 ± 0.41 ^{Ac}	10.13 ± 0.28 ^{Aa}	9.16 ± 0.60 ^{Ba}	9.62 ± 0.46 ^{Abc}	9.81 ± 0.61 ^{Bc}
Distilled water	6.8 ± 0.4 ^{Ad}	7.0 ± 0.1 ^{Ad}	7.0 ± 0.4 ^{Ad}	7.1 ± 0.1 ^{Ac}	6.6 ± 0.2 ^{Ad}	6.9 ± 0.7 ^{Ad}
(B) Calcium released (ppm)						
MTA Plus	43.4 ± 13.0 ^{Aa}	22.9 ± 7.1 ^{Ba}	15.8 ± 4.3 ^{Ba}	11.2 ± 5.6 ^{Bab}	14 days	28 days
MTA Plus gel	119.0 ± 32.4 ^{Ab}	85.5 ± 36.9 ^{Bb}	20.5 ± 7.7 ^{Ca}	12.7 ± 3.6 ^{Cab}	8.9 ± 2.2 ^{Ba}	7.7 ± 4.3 ^{Ba}
ProRoot MTA	24.1 ± 3.9 ^{Ac}	21.7 ± 12.0 ^{Aa}	16.8 ± 4.3 ^{Aa}	15.4 ± 5.9 ^{Aa}	11.4 ± 3.0 ^{Ca}	19.5 ± 12.4 ^{Cb}
Dycal	24.7 ± 6.3 ^{Ac}	28.4 ± 9.18 ^{Aa}	38.5 ± 8.6 ^{Bb}	17.6 ± 5.2 ^{Ca}	26.5 ± 7.7 ^{Ab}	16.4 ± 1.4 ^{Ab}
Distilled water	1.0 ± 0.8 ^{Ad}	0.6 ± 1.1 ^{Ac}	0.8 ± 0.7 ^{Ac}	1.6 ± 0.8 ^{Ab}	18.61 ± 3.9 ^{Cb}	16.2 ± 5.5 ^{Cb}
(C)						
Exterior volume (V, cm ³)						
MTA Plus	0.0941 ± 0.0056 ^a	0.03801 ± 0.0017 ^a	0.0569 ± 0.0033 ^a	40.34 ± 1.08 ^a	24.85 ± 1.41 ^a	18.51 ± 0.72 ^a
MTA Plus gel	0.1063 ± 0.0095 ^a	0.0424 ± 0.0068 ^b	0.06475 ± 0.0082 ^b	39.30 ± 6.33 ^a	26.42 ± 6.26 ^a	14.62 ± 3.67 ^b
ProRoot MTA	0.1072 ± 0.0068 ^a	0.0318 ± 0.0025 ^c	0.0754 ± 0.0046 ^c	29.36 ± 0.75 ^b	14.73 ± 0.53 ^b	10.79 ± 0.50 ^c
Dycal	0.1053 ± 0.0060 ^a	0.0097 ± 0.0004 ^d	0.0961 ± 0.0064 ^d	9.04 ± 0.41 ^c	5.01 ± 0.26 ^c	4.94 ± 0.29 ^d
Volume of open pores (Vop, cm ³)						
MTA Plus						
MTA Plus gel						
ProRoot MTA						
Dycal						
Volume of impervious portion (Vip, cm ³)						
MTA Plus						
MTA Plus gel						
ProRoot MTA						
Dycal						
Apparent porosity (Vop/V %)						
MTA Plus						
MTA Plus gel						
ProRoot MTA						
Dycal						
Water sorption (%)						
MTA Plus						
MTA Plus gel						
ProRoot MTA						
Dycal						
Solubility (%)						
MTA Plus						
MTA Plus gel						
ProRoot MTA						
Dycal						

Different letters represent statistically significant differences (2-way repeated measures analysis of variance followed by the Student-Newman-Keuls test, $P < .05$) in the same line (capital letters) or in the same column (small letters) ($n = 10$ per group).

peak for the MTA Plus material became obscured by a fine precipitate covering the surface, which appeared less dense than the MTA Plus/gel sample. The Ca/P values after 7 and 28 days in HBSS were 5.32 and 2.1, respectively.

Freshly mixed ProRoot MTA had a fine, granular surface with EDX peaks for major phases with calcium and silicon and lesser peaks for bismuth (10% wt) and aluminum (0.6% wt). After soaking, traces of sodium, chlorine, and phosphorous were observed, and the bismuth peak decreased. A high Mg peak was detected at 7 days and thereafter. At 28 days in HBSS, the surface was coated by globular calcium phosphorous precipitates, nonuniformly distributed, with a Ca/P ratio of 1.84; however, bismuth was still detectable.

Fresh Dycal exhibited a uniform surface with prominent calcium, phosphorous, tungsten, titanium, zinc, sulfur, and carbon. The Ca/P ratio of 3.75 reflected the organic components (ie, Ca[OH]₂, calcium phosphate, and calcium tungstate) known to be present in Dycal. Carbon and tungsten were obscured at 7 days, whereas the calcium and phosphorous peaks increased in intensity, and sodium, magnesium, and chlorine peaks were detected. Overall, the tungsten, titanium, zinc, sulfur, and carbon peaks decreased at longer soaking times. At 28 days, the layer of Ca/P precipitates was insufficient to mask titanium and zinc; the Ca/P ratio was 1.64.

Discussion

These *in vitro* tests of the 2 MTA products and Dycal highlight the similarities of the MTA products and the contrast with Dycal, a Ca(OH)₂-based product. All 3 materials released calcium and hydroxide ions. The MTA products are known to form Ca(OH)₂ in a calcium silicate hydroxide matrix. EDX disclosed calcium, silicon, aluminum, and bismuth in the MTAs, indicative of calcium silicates, calcium aluminate, and bismuth oxide. The MTA products appeared to contain bismuth oxide, and the Dycal material was deduced to contain its stated calcium tungstate zinc oxide and titanium dioxide for radiopacity. MTA Plus had a composition similar to ProRoot MTA, but MTA Plus with water had a small peak for sulfur, indicating calcium sulfate, which was also presumed to be present in ProRoot MTA but not observed with it or MTA Plus and gel.

The beneficial release of Ca(OH)₂ diminished for all materials over 1 month. The precipitated calcium phosphate layer reduced the diffusion of calcium or hydroxide ions. MTA Plus with gel had high calcium release and pH. MTA Plus and gel slightly reduced the porosity, sorption, and solubility. ProRoot MTA had a calcium release lower than previously reported (15, 16) despite the same methodology but was similar to Dycal. The Dycal data were similar to that reported in other studies (eg, low solubility [4.94%]) (15, 16,18–20).

High porosity, solubility, and water sorption were observed for the MTA compounds. However, these measurements were initiated after as little as 55 minutes after mixing. The solubility was calculated as weight loss, with a method different from the ISO 6876 method for root canal sealers. The present solubility values are much higher than measured in other studies in which the samples tested set for 21–24 hours at 37°C and 98%–100% relative humidity before immersion in water (21–23). In these tests, the high surface to volume ratio also differs from the clinical situation in a root-end filling in which the surface to volume ratio is lower. The solubility of ProRoot MTA (14.7%) measured here seems inconsistent with its excellent clinical performance; however, the solubility is reduced in biological fluids (15, 24) by the rapid formation of a calcium phosphate layer. The ongoing hydration reactions of dicalcium silicate (lasting about 28 days) undoubtedly interfered with these measurements.

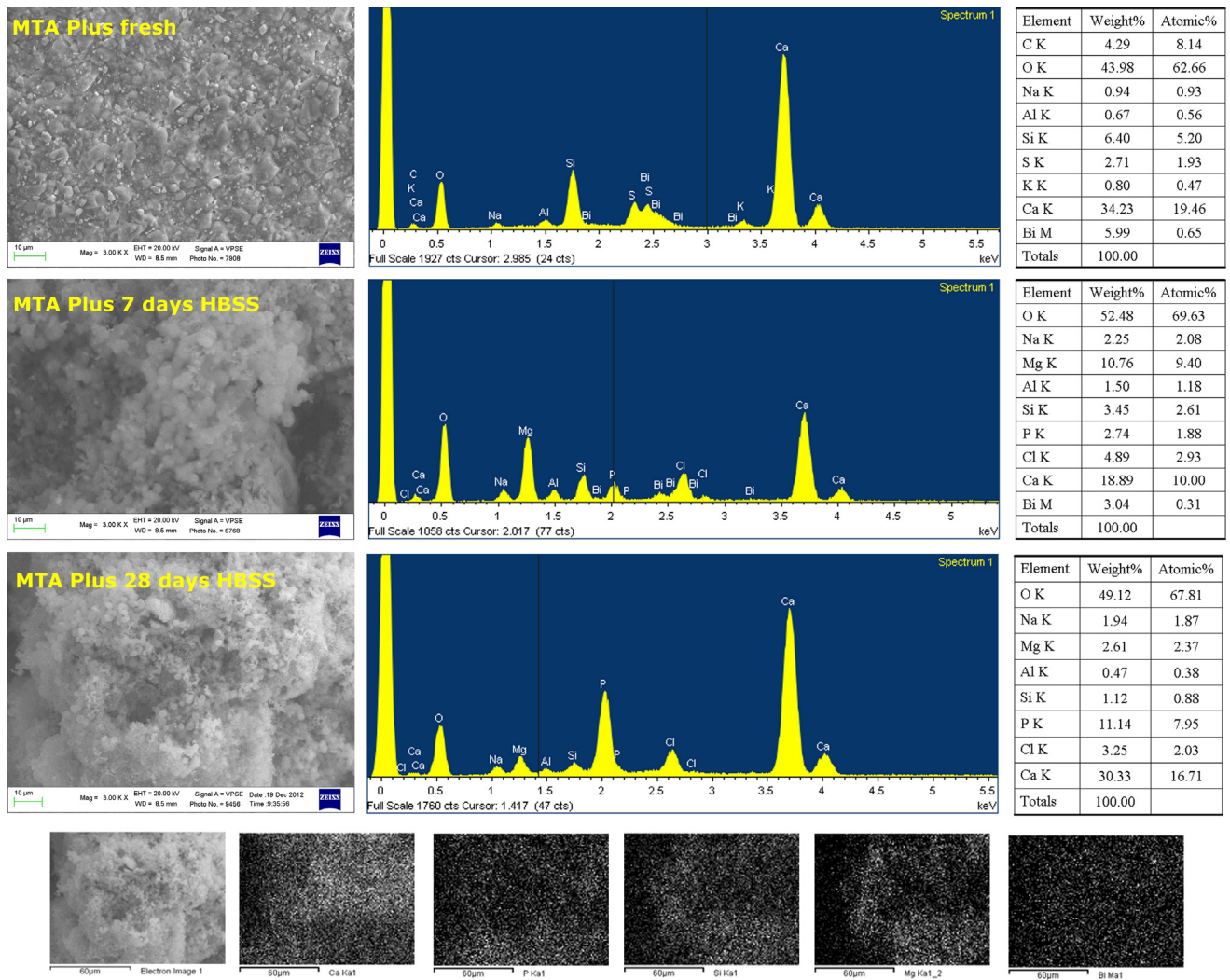


Figure 1. ESEM images with EDX microanalysis evaluating the chemical composition (spectra) and the element distribution (elemental mapping) of MTA Plus freshly prepared and soaked for 7 and 28 days in HBSS. The HBSS solution contained, 1.27 mmol/L Ca^{2+} , 144.7 mmol/L Cl^- , 5.8 mmol/L K^+ , 141.6 mmol/L Na^+ , 0.81 mmol/L Mg^{2+} , 4.17 mmol/L HCO_3^- , 0.81 mmol/L SO_4^{2-} , 0.44 mmol/L H_2PO_4^- , and 0.336 mmol/L HPO_4^{2-} .

Solubility is less for calcium silicates after a longer setting time. *In vivo*, the exposed surface to volume ratio is less than tested here.

The elution of the calcium and hydroxide ions apparently initiated the formation of calcium phosphate phases but also increased the porosity. The Dycal material appeared less bioactive; its precipitate did not mask the underlying material from the electron beam, suggesting/confirming a thin apatite layer. This was true to a lesser degree for the ProRoot MTA. Both MTA products produced thicker precipitates with a higher Ca/P ratio exceeding 1.67, indicating a coprecipitation of calcium carbonate formed from the $\text{Ca}(\text{OH})_2$. MTA Plus formed a thick calcium phosphate layer (good bioactivity) after soaking in simulated body fluid forming large calcium phosphate-based spherules. This material has shown dentin remineralization in the presence of SBF (25). The high calcium and OH ion releases are connected with the formation of calcium phosphate deposits.

MTA Plus had a higher ion release than ProRoot MTA and Dycal; the use of the MTA Plus gel enhanced the initial calcium release and raised the pH. The finer calcium silicate powder may explain the higher ion release, water sorption, porosity, and solubility of MTA Plus compared with ProRoot MTA. Both MTA products were more water soluble and water sorptive than Dycal and more bioactive.

The ability to release calcium ions able to diffuse through dentin (8) and inside the surrounding tissues is a key factor for successful endodontic and pulp capping therapies because of the action of calcium on the differentiation of mineralizing cells as dental pulp cells (26, 27), cementoblasts (28), osteoblasts (29, 30), periodontal fibroblasts (31, 32), mesenchymal stem cells (33), and hard tissue mineralization. Alkaline pH values accelerate apatite nucleation because apatite solubility decreases and OH^- may be a component of apatite (17). Also, hydroxide ions stimulate the release of alkaline phosphatase and bone morphogenetic protein 2, which participate in the mineralization process (31). The MTA cements' apatite-forming ability has favorable clinical implications as root canal filling materials in association with gutta-percha because their sealing is improved with time by the deposition of calcium phosphates at the interface and inside the dentinal tubules (34).

The mechanism of apatite formation on calcium silicate MTA cements in phosphate-containing solutions was summarized by Gandolfi et al (1) in 11 steps. The growth of a layer of apatite is an ideal environment for stem cell and osteoblast differentiation and colonization to support new bone formation. Apatite together with the epigenetic signals correlated to ion release may well explain

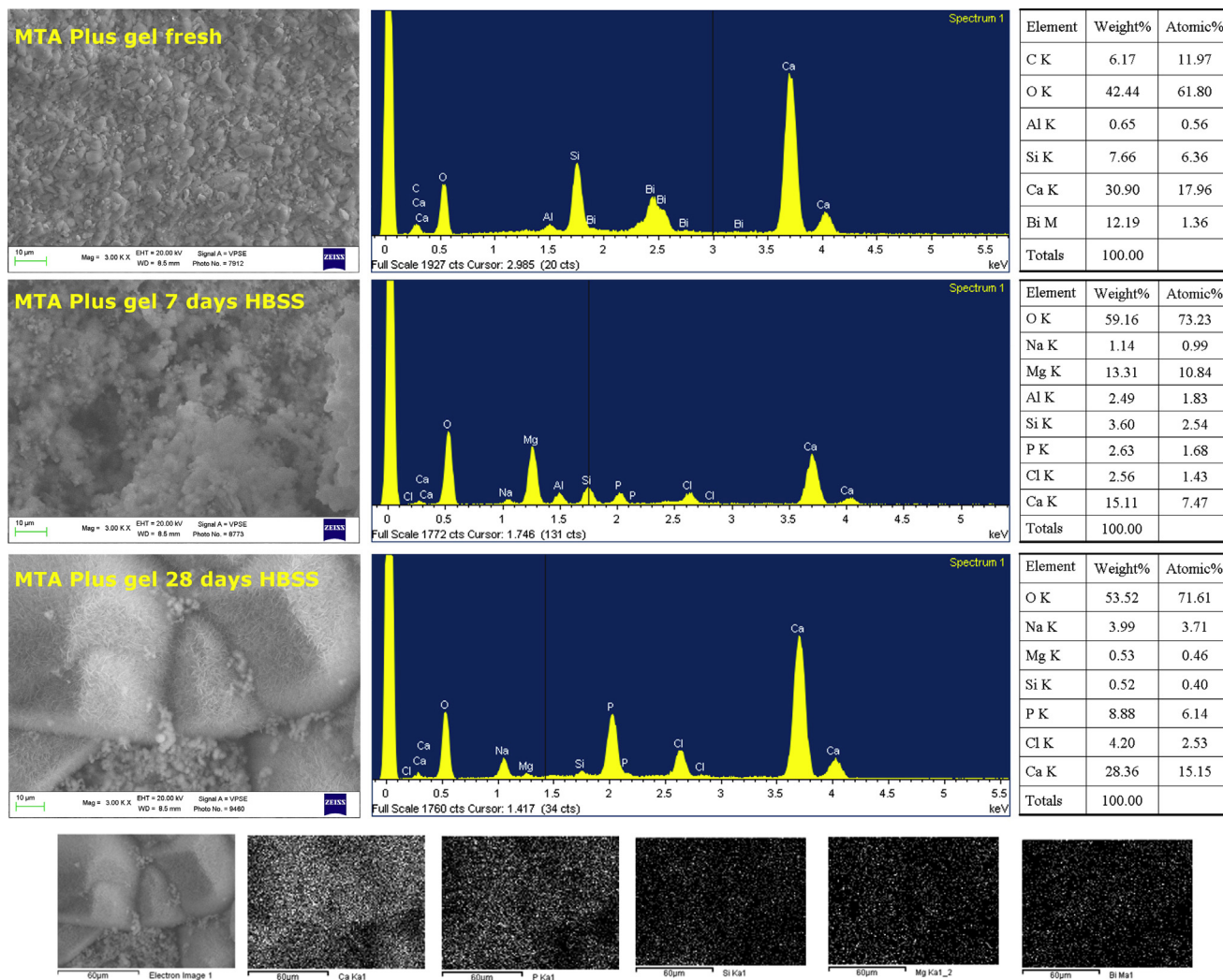


Figure 2. ESEM/EDX analysis of MTA Plus gel soaked in HBSS.

the excellent clinical outcomes of MTA cements (35). Moreover, the apatite-forming ability may provide clinical advantages by improving their sealing by the deposition with the time of calcium phosphates at the interface and inside the dentinal tubules of the root canal when MTA cements are used as root canal filling materials in association with gutta-percha (34).

MTA Plus had a prolonged capability to release calcium and increase the local pH in comparison with ProRoot MTA. These ion-releasing properties are interlinked with its noticeable porosity, water sorption, and solubility and with the formation of a calcium phosphate layer. For clinicians, MTA Plus represents a lower-cost, bioactive tricalcium silicate material as a convenient alternative to the conventional calcium silicate MTA-like cements.

Acknowledgments

Dr Primus is the inventor of MTA Plus and white ProRoot MTA. She is not part of Prevest Denpro or Dentsply and did not participate in the data collection nor statistical analysis.

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The authors deny any conflicts of interest related to this study.

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