Chemomechanical Properties and Biocompatibility of Various Premixed Putty-type Bioactive Ceramic Cements

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ABSTRACT

Introduction: This study aimed to evaluate the chemomechanical properties and biocompatibility of recently introduced premixed putty-type bioactive ceramic cements (PPBCs). **Methods:** Including ProRoot MTA (PMTA) as a control, BC RRM fast-set putty (BCPT), Well-Root PT (WRPT), One-Fil PT (OFPT), and Endocem MTA premixed (ECPM) were compared to evaluate setting time, radiopacity, pH change, and microhardness. Biocompatibility on human dental pulp cells was compared using CCK-8 assay. Mineralization potential was evaluated using alkaline phosphatase activity, Alizarin Red S (ARS) staining, and quantitative real-time polymerase chain reaction with odontogenic gene marker. For data analysis, 1-way analysis of variance and Tukey's post hoc test were used at the significance level of 95%. **Results:** Among the PPBCs, BCPT presented the longest (552 ± 27) setting time (minutes) and others showed significantly shorter time than PMTA (334 ± 22) ($P < .05$). WRPT (6.20 ± 0.54) and OFPT (5.82 ± 0.50) showed significantly higher radiopacity values (mmAl) and others showed similar value compared with PMTA ($P > .05$). All PPBCs showed high alkaline pH from fresh materials and tended to increase according to time periods from 30 minutes to 12 hours. ECPM showed the highest value of microhardness (81.62 ± 5.90), WRPT showed similar, and others showed lower than PMTA ($P < .05$). All PPBCs showed biocompatibility in CCK-8 assay. All PPBCs showed similar or better value compared with PMTA in ALP and ARS staining, and ALP and DSPP marker expression ($P < .05$). **Conclusions:** The PPBCs showed clinically acceptable chemomechanical properties and favorable mineralization potential. *(J Endod 2023;49:1713–1721.)*

KEY WORDS

Biocompatibility; mineralization potential; microhardness; premixed putty-type bioceramics; radiopacity; setting time

SIGNIFICANCE

The recently introduced premixed putty-type bioceramics, which have the advantages of uniform consistency and user-friendly manipulation, showed clinically acceptable chemomechanical properties and favorable mineralization potential.

Since bioactive materials were introduced in endodontics, they have been applied in various clinical uses such as root-end surgery, pulp capping, pulpotomy, root perforation repair, apexogenesis, and regenerative endodontic procedures[1]. Mineral trioxide aggregate (MTA) was the first material in this category and proved to be a bioactive material that is osteo-conductive, osteo-inductive, and biocompatible[2,3]. With its biocompatible characteristics, favorable clinical outcomes have been reported in vital pulp therapy, apical surgery, and perforation repair[4-6]. Representatively, pulp capping using MTA has reported superior and predictable outcomes compared with using calcium hydroxide, leading to MTA being considered the first choice as a capping material[5,7].

However, a number of concerns related to MTA have been addressed, such as the prolonged setting time and discoloration, and they are sensitive to manipulate and difficult to handle[1]. To overcome these drawbacks, a range of bioactive ceramic materials have been developed continuously. Therefore, rapid-setting cements containing fine-size particles or calcium chloride as an accelerator in the liquid have been introduced, such as Endocem MTA (Maruchi, Wonju, Korea) and Biodentine (Septodont, Saint-Maur-des-fossés, France)[8,9]. In addition, several products currently have become available in which zirconium oxide has been used as a radiopacifier, instead of bismuth oxide, which is suspected of causing discoloration[10].

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Last, mixing and handling issues are the significant drawbacks of MTA. The powder/liquid system is so technique sensitive that it can produce inconsistent results even when mixed according to the manufacturer's instructions. Indeed, any change in the powder/liquid ratio or uneven mixing procedure could alter the physicochemical properties of cements[11,12]. In addition, some surveys reported that dentists are avoiding using MTA because it is difficult to handle[13,14]. In 2007, iRoot SP injectable root canal sealer (Innovative BioCeramix, Inc., Vancouver, Canada) was introduced as the first premixed, ready-to-use calcium silicate–based material. The next year, a putty-type premixed bioactive ceramic product was available from Brasseler USA as EndoSequence Root Repair Materials (Brasseler, Savannah, GA)[15].

Recently, various premixed putty-type bioactive ceramic cements (PPBCs) have been developed and released into the market in the form of capsules, syringes, and syringes with needles. EndoSequence BC RRMTM fast-set putty (BCPT) (Brasseler) contains the same characteristics as previous BC RRM-putty but with a new fast-set chemistry and is even more resistant to washout[16]. Well-Root PT (WRPT) (Vericom Co., Chuncheon, Korea) is an insoluble and radiopaque material based on a calcium aluminosilicate composition[17]. It is given in capsules, unlike others provided in syringes, so it is placed using a capsule dispenser gun. One-Fil PT (OFPT) (Mediclus Co., Cheongju, Korea) is a recently released PPBC that is based on tricalcium silicate compounds composition requiring the presence of water to set[18]. Endocem MTA premixed regular (ECPM) (Maruchi) has rapid sol-gel changes for compaction and excellent physical properties and biocompatibility comparable to that of powder-type Endocem MTA[19].

PPBCs have the advantages of uniform consistency and user-friendly manipulation. To take priority consideration ahead of the traditional powder/liquid types of bioactive ceramic cements, a large number of studies are required to support that they have an additional advantage of easy handling with characteristics comparable to MTA. Therefore, this study aimed to evaluate the chemomechanical properties and biocompatibility of recently introduced PPBCs—BCPT, WRPT, OFPT, and ECPM, including ProRoot MTA (PMTA) (Dentsply Tulsa, Tulsa, OK) as a control. The null hypothesis is that PPBCs show comparable characteristics compared with PMTA, and can be acceptable and more efficiently used in the clinic.

MATERIALS AND METHODS

The chemomechanical and biocompatible properties of PMTA, BCPT, WRPT, OFPT, and ECPM were evaluated. Table 1 lists the manufacturer and composition of the materials.

Setting Time

As defined in this ISO 6876[20], a gypsum mold was used for the PPBCs that require moisture for setting. Samples of PPBCs (n = 15 per group) were placed in a gypsum mold of 10-mm diameter and 2-mm height in an incubator (C-IN Incubator; Changshin-lab, Pocheon, Korea) at 37°C and a 95% humidity level. A Gilmore-type needle of 100-g weight and 2-mm diameter was carefully placed vertically on the surface of the PPBC sample. Based on the preliminary test data, the measurements were repeated every 5 minutes an hour after the material was filled in the mold. When the needle did not make a visible indentation on the material surface was recorded as the setting time.

Radiopacity

For the measuring of radiopacity, aluminum (Al) step wedge with variable thickness from 1 to 10 mm in 1-mm increments was used according to the ISO 6876[20]. Each PPBC was placed in a metallic ring mold of 8-mm diameter and 1-mm height. The samples (n = 15 per group) were stored in an incubator at 37°C with a humidity of 95% for 24 hours until the material was completely set. Radiographs of the sample and aluminum step wedge were taken using a radiographic unit (DIOX-602; Digimed, Seoul, Korea) operating at 60 kV and 2 mA and exposure for at 0.08 seconds. The distance between the tube and sensor was 10 cm. After importing the digitized radiographs into ImageJ software version 1.53 (National Institutes of Health, Bethesda, MD), the mean gray values of the sealer sample and aluminum step wedge were compared.

Microhardness

Each PPBC wase placed in a Teflon mold of 5-mm diameter and 2-mm height. The samples (n = 5 per group) were stored in an incubator at 37°C with a humidity of 95% for 24 hours until the material was completely set. Vickers microhardness was measured using a hardness testing machine (MiCAT HM-200: Mitutoyo, Kanagawa, Japan) under 0.3 kgf loading. From each sample, 3 spots were measured, and 15 data were attained from each PPB for statistical comparison.

pH Analysis

The samples from the material package were inserted into the Teflon tube of 6-mm diameter and 2-mm height and classified as fresh samples, whereas those kept in the incubator to set were classified as set samples.

Both fresh and set samples were placed in distilled water in a polypropylene conical tube and stored at 37°C for the duration of the analysis. The pH of the solution was measured using a digital pH meter (STARTER 2100; Ohaus, Greifensee, Switzerland) at 10 minutes, 1 hour, 3 hours, and 12 hours directly after material manipulation for fresh samples; initial, 12 hours, 1 week, and 4 weeks after setting for set samples. Ten samples from each group were measured for pH at each period.

Ion Releasing Profiling

The released ions from the materials were identified using inductively coupled plasma optical emission spectrometry (ICP-OES) (Optima 4300 DV, PerkinElmer, Shelton, CT). The materials were molded into discs with a diameter of 2 mm and a height of 2 mm, and set under 37°C and 100% humidity for 1 hour. The set materials were then immersed in 10 mL de-ionized water for 1 day, and the solutions were used for leached ion identification (n = 3).

Human Dental Pulp Cell Isolation and Cell Culture

Human dental pulp cells (hDPCs) were collected from the third molar of patients from the Department of Oral Surgery, Dankook University Hospital (DKUDH IRB 2019-01-006). The cells were cultured in α-MEM (Gibco; Thermo Fisher Scientific, Waltham, MA) supplemented with 10% fetal bovine serum, 200 μM L-glutamine (Gibco) and gentamycin (Gibco) at 37°C, 5% CO_2, and 95% humidity. Generally, the cells at passage 4-5 were used for further experiments.

Preparation of Extract Media for Cell Treatment

PMTA and 5 PPBCs were used (Table 1). After PMTA was mixed according to the manufacturer's instructions, all materials were placed into polyvinyl chloride disc molds. The discs containing the materials hardened at room temperature for 1 hour, then were immersed in α-MEM for 24 hours. The extracts were filtered using a 0.22-μm pore-size syringe filter (MilliporeSigma, St. Louis, MO).

Cell Viability Assay

Cell counting kit-8 (CCK-8; DOJINDO, Kumamoto, Japan) was used to quantitatively evaluate the proliferation of hDPCs affected by material extracts. Briefly, the cells were treated with extracts of materials at 12.5%～100% various concentrations. CCK-8 working solution was added to each well and incubated at 37°C for 1 hour. The absorbance of living cells was measured at 450 nm using a microplate reader (Bio Rad Laboratories; Hercules, CA).

Alkaline Phosphatase and Alizarin Red S Staining

When cells reached 90% to 100% confluence, they were induced with extraction medium containing osteogenic supplements (100 μM ascorbic acid 2-phosphate, 10 mM β-glucerophosphate, and 10 nM dexamethasone). Cells were cultured for 7 days at 37°C, then fixed with 4% paraformaldehyde. Alkaline phosphatase (ALP) staining was tested by NBT/BCIP (Sigma-Aldrich, St. Louis, MA) substrate solution for 30 minutes. Cells were rinsed 3 times with distilled water to completely remove the redundant stains. ALP-positive cells and mineralized nodules were imaged with a digital scanner. Alizarin Red S (ARS) staining was performed 14 days after induction. Extracellular mineralized nodules were stained by ARS solution (pH 4.2). For ARS staining, the cells were washed twice with phosphate-buffered saline (PBS) and fixed in 70% EtOH at room temperature for 10 minutes. Then, the cells were washed twice with PBS and stained with 2% ARS solution (pH 4.2) at room temperature for 30 minutes in the dark. Cells were rinsed 3 times with distilled water to completely remove the redundant stains. ALP and ARS were eluted from stained odontoblasts with 200 μL 10% (w/v) cetylpyridinium chloride (CPC) (Sigma-Aldrich) in an aqueous 0.01 M Na2HPO4/NaH2PO solution (pH 7) for 1 hour. One hundred fifty microliters was transferred on a 96-well plate, and absorbance was measured at 560 nm. Ten percent (w/v) CPC in an aqueous 0.01 M Na2HPO4/NaH2PO4 solution was used as blank.

Quantitative Real-Time Polymerase Chain Reaction

To evaluate odontogenic differentiation in hDPCs, quantitative real-time polymerase chain reaction was used to determine the expression of odontogenic genes (ALP, DMP1, and DSPP). After 1, 3, and 7 days of differentiation, cells were washed with 1 × PBS. Total RNA was isolated using Ribospin (GeneAll, Seoul, Korea) according to the manufacturer's protocol and 1 μg RNA was reverse transcribed into complementary DNA by Accupower RT Premix for quantitative real-time polymerase chain reaction (BIONEER, Daejeon, Korea). Quantitative real-time polymerase chain reaction was performed with SensiMix SYBR Hi-ROX Kit (Bioline, Memphis, TN) on an ABI7500 quantitative PCR instrument (Applied Biosystems, Waltham, MA). The primer sequences are detailed in Table 2.

Statistical Analysis

Statistical analysis of data was performed using the Prism 5.0 software package (GraphPad, Boston, MA) with a significance level of 95%. One-way analysis of variance and Tukey's multiple-comparison tests were performed to compare the means across the 5 groups.

TABLE 1 - Materials Used in the Present Study

Product	Code	Manufacturer	Composition*
ProRoot MTA	PMTA	Dentsply Tulsa, USA	Tricalcium silicate, dicalcium silicate, tricalcium aluminate, gypsum, tetracalcium aluminoferrite, bismuth oxide
Endosequence BC RRM Fast-Set Putty	BCPT	Brasseler, USA	Tricalcium silicate, zirconium oxide, tantalum pentoxide, dicalcium silicate, calcium sulfate
Well-Root PT	WRPT	Vericom, Korea	Calcium aluminosilicate compound, zirconium oxide, Thickening agent
One-Fil PT	OFPT	Mediclus, Korea	Tricalcium silicate compounds, zirconium oxide, hydrophilic polymer (thickening agents)
Endocem MTA Premixed	ECPM	Maruchi, Korea	Zirconium dioxide, calcium silicate, calcium aluminate, calcium sulfate, dimethyl sulfoxide, thickening agent

*The compositions were according to the available information included in the manufacturer's material safety data sheets.

RESULTS

Setting Time, Radiopacity, and Microhardness

Generally, compared with PMTA, PPBCs tended to have shorter setting time (minutes), higher radiopacity (mmAl), and lower microhardness (Table 3). Except for BCPT presenting the longest setting time (552 ± 27), all PPBCs showed significantly shorter setting times than PMTA ($P < .05$). WRPT (6.20 ± 0.54) and OFPT (5.82 ± 0.50) showed higher values of radiopacity than PMTA ($P < .05$), and BCPT and ECPM showed values similar to PMTA (4.82 ± 1.02). ECPM showed the highest value of microhardness (81.62 ± 5.90), which is higher than PMTA (53.47 ± 4.27, $P < .05$). WRPT, BCPT, and OFPT showed lower values than PMTA in order ($P < .05$).

pH Change

The pH value of all fresh samples increased gradually from immediately after application until 6 hours (Fig. 1*A*). PMTA showed the highest value of pH all the time, with presenting above 11 ($P < .05$), whereas OFPT and WRPT tended to have relatively lower pH than the PMTA all the measured time. The pH of all set samples showed a relatively low pH, under 10; however, gradually increased according to the time period and stabilized at approximately 11.4 or 11.7 at 4 weeks (Fig. 1*B*).

Ion Release

The calcium, silicon, and zirconium ion release profile of each material was identified (Fig. 2). Calcium ion exhibited greater release than Si and Zr. All PPBCs showed similar release of Ca compared with PMTA; however, silicate ions exhibited less release. OFPT showed similar silicate release compared with PMTA. Zirconium ion was not identified in PMTA and BCPT.

Cell Cytocompatibility

To reproduce the clinical environment, the cell viability was determined with 1-hour setting extract. All materials showed 100% cell viability to hDPCs until 72 hours (Fig. 3*A*). Based on the results, the concentration of the extracts used in subsequent experiments was determined to be 1:2 in hDPCs.

TABLE 2 - The Primer Lists Used for Polymerase Chain Reaction Analysis

Gene name	Sequence (5′ ► 3′)
GAPDH_Fwd	CCAGAACATCATCCCTGCCTCT
GAPDH_Rev	GACGCCTGCTTCACCACCTT
DMP-1_Fwd	CAGGAAGAGGTGGTGAGTGAGT
DMP-1_Rev	TGGATTCGCTGTCTGCTTGCT
DSPP_Fwd	GGGAATATTGAGGGCTCCAA
DSPP_Rev	TCATTGTGACCTGCATCGCC
ALP_Fwd	ACCATTCCCACGTCTTCACATTT
ALP_Rev	AGACATTCTCTCGTTCACCGC

Mineralization Potential

Except for BCPT, all PPBCs showed similar ALP activity and calcium deposition compared with PMTA (Fig. 3*B* and *C*). The overall gene expression of odontogenic markers (ALP, DMP1, and DSPP) gradually increased in all groups (Fig. 3*D*). At 7 days, BCPT and PMTA had a higher upregulated expression of ALP and DMP1 than the induction media group, respectively ($P < .05$). Collectively, PPBs showed comparable mineralization capacity to PMTA; furthermore, BCPT was superior to PMTA via ALP, ARS staining, and ALP marker expression.

DISCUSSION

In the present study, the investigation was focused on the characteristics required for clinical application environments such as vital pulp therapy. These environments involve direct contact with vital/pulp tissue under the final restoration.

Setting time is an influencing factor not only for the procedure but also for the treatment outcome. A long setting time was one of the major drawbacks of MTA[21], which may disadvantage its application in supracrestal areas or compromise the integrity of the seal by early occlusal forces[22]. Therefore, PPBCs in this study may benefit clinical application by having a shortened setting time (less than 130 minutes). However, they showed extended setting time compared with the given setting time provided by the manufacturers (Table 3)[16-19], which might be related to the decision point of how much delicate and sensitive to confirm that there is completely no indentation by the Gilmore needle. BCPT is the renewed "fast-set" product suggesting approximately 25 minutes; however, it rather showed longer than PMTA.

According to the manufacturer, PPBC material is produced with a nanosphere, the largest particle size of 0.35 mm, with approximately 50% of the particles being nano (1×10^{-3} μm) in size[23]. Therefore, they can enter the dentinal tubules and initiate the hydration process with the moisture in the dentinal tubules. It is convenient that no additional moisture supply is required due to the small particles. Especially, PPBSc can easily initiate hydration via contact with the tissue fluid and blood. However, the setting time in the clinic may be inconstant, as shown in this study, because there are more variables in the clinical environments (situations such as apical surgery, perforation repair, pulpotomy, and pulp revascularization), such as the degree of wettability of dentin and humidity of the root canal lumen. Setting time is closely related to the hydration reaction, which influences physical properties[22]. Therefore, further studies are required to control the setting time more consistently.

Dental materials should have sufficient radiopacity for the radiographic diagnosis and evaluation. Based on ISO 4049[24] and 9917-1[25], the restorative material must have a radiopacity equal to or greater than the radiopacity of aluminum at the same thickness. The ISO 6876 standard recommended the value of 3 mmAl to be distinguishable from dentin. In addition, it has been reported that it should have a slightly higher radiopacity than that of enamel in order to detect secondary caries[26]. In this study, all PPBCs achieved the minimum requirement of 3 mmAl and also showed a higher value than PMTA. Considering that zirconium is the main radiopacifier, the effects from the different ratios of ingredients would be the main reason for the difference of the radiopacities.

Regarding microhardness, all PPBCs except for ECPM showed similar and lower value than that of PMTA. Microhardness is defined as the resistance to surface indentation. Considering the PPBs are generally located under the final restoration such as PMTA does and the possibility of retreatment, it would be acceptable for the microhardness similar to PMTA. Microhardness can also be used as an indicator of the hydration process of hydraulic cements and their setting process[27,28]. ECPM showed that 1.5 times higher than PMTA can be considered to have a fast hydraulic reaction and high crystal structure stability. Considering that the setting process continues even after the initial setting, the value of microhardness will increase with time, consistent with the previous studies[22,29].

The high alkaline pH of the bioactive ceramic cements is partially responsible for this antibacterial nature and mineralization potential. Therefore, it is advantageous to maintain an alkaline pH for a long time after application. In this study, both fresh and set samples showed increasing pH over time, which is consistent with the previous study[30]. Ion release occurs more actively in a material that is not fully hardened, and high pH from fresh samples has resulted from more calcium and hydroxyl ions released. Based on the results, we can expect that fresh cement could promote pulp tissue healing and mineralized tissue formation[31].

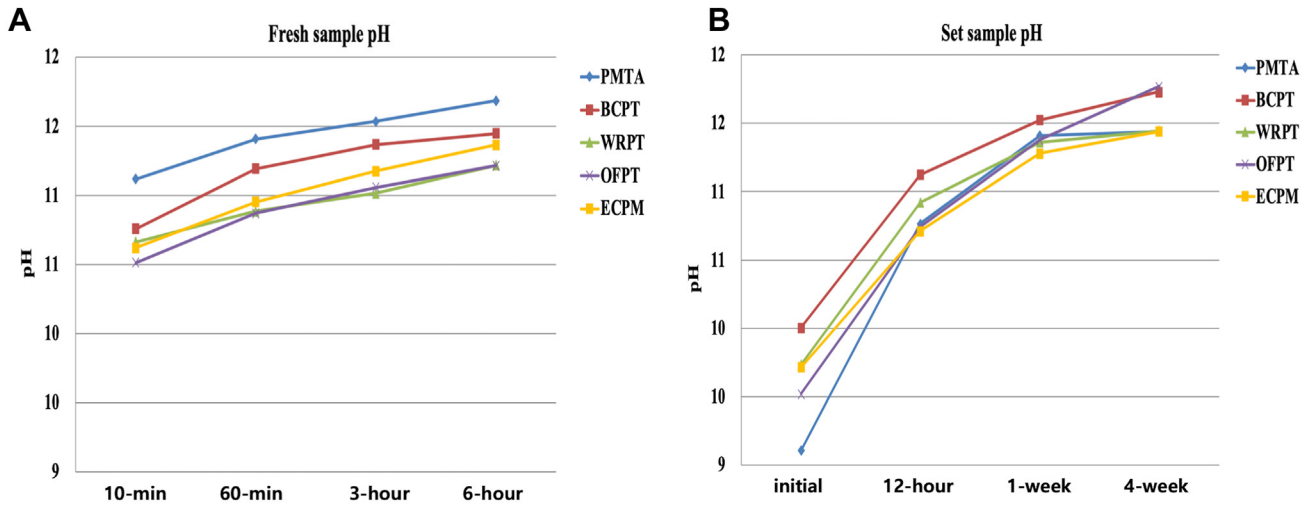
Ion releases of hydraulic cements have been correlated with biological properties, as they stimulate the differentiation potential of dental pulp cells. It has been reported that tricalcium silicate–based biomaterials such as MTA influence the intracellular Ca^{2+} dynamics via calcium release, resulting in the cellular

TABLE 3 - Physicochemical Value of Setting Time (min), Radiopacity (mmAl), and Microhardness (Mean ± Standard Deviation)

	ProRoot MTA	Endosequence BC RRM Fast-set putty	Well-root PT	One-Fil PT	Endocem MTA premixed
Setting time	240*	20*	25*	30*	4.2*
	334 ± 22^{b}	552 ± 27^{a}	128 ± 9^{c}	130 ± 9^{c}	94 ± 12^{d}
Radiopacity	4.8 ± 1.0^{b}	5.5 ± 0.8^{ab}	6.2 ± 0.5^{a}	5.8 ± 0.5^{a}	4.8 ± 0.7^{b}
Microhardness	53.5 ± 4.3^{b}	42.7 ± 6.8^{cd}	50.5 ± 5.2^{bc}	35.8 ± 3.6^{d}	81.6 ± 5.9^{a}

^{a,b,c,d}Different superscript letters mean significant difference between groups ($P < .05$).

*The setting time provided by the manufacturer.[16-19]



	10-min	60-min	3-hour	6-hour
PMTA	11.12±0.42 [a]	11.41±0.31 [a]	11.54±0.26 [a]	11.69±0.14 [a]
BCPT	10.76±0.12 [b]	11.19±0.11 [b]	11.37±0.05 [a]	11.45±0.13 [b]
WRPT	10.66±0.30 [b]	10.89±0.18 [c]	11.02±0.13 [b]	11.22±0.11 [c]
OFPT	10.51±0.21 [b]	10.87±0.11 [c]	11.06±0.08 [b]	11.22±0.13 [c]
ECPM	10.62±0.14 [b]	10.9±0.11 [c]	11.18±0.13 [b]	11.37±0.23 [bc]

	initial	12-hour	1-week	4-week
PMTA	9.11±1.03 [a]	10.77±0.11 [b]	11.41±0.14 [ab]	11.44±0.17 [b]
BCPT	10.00±0.55 [a]	11.13±0.22 [b]	11.53±0.019 [a]	11.73±0.26 [a]
WRPT	9.74±0.35 [a]	10.92±0.16 [ab]	11.36±0.07 [ab]	11.45±0.23 [b]
OFPT	9.52±0.82 [a]	10.74±0.26 [b]	11.38±0.04 [ab]	11.77±0.16 [a]
ECPM	9.72±1.09 [a]	10.71±0.36 [b]	11.28±0.16 [b]	11.44±0.16 [b]

FIGURE 1 – pH change of (*A*) fresh and (*B*) set samples. Different letters ([a,b,c] in the embedded tables) indicate significant differences between groups ($P < .05$). BCPT, BC RRM fast-set putty; ECPM, Endocem MTA premixed; OFPT, One-Fil PT; WRPT, Well-Root PT.

differentiation and mineralization potential of hDPSCs[32]. This study revealed that all PPBCs showed a calcium release comparable to PMTA. BCPT tended to release the highest amount of calcium, which can explain the high mineralization potential shown in ALP and ARS staining. Silicate ions are known to enhance osteogenic differentiation and stimulate angiogenesis[33,34]. In this study, high silicate release was observed in PMTA and OFPT, and related studies are needed to confirm the effect.

The biological characteristics of PPBCs are essential for pulp therapy, especially in cases in which the vital pulp tissue is exposed and should be covered with dental materials. PPBCs have a similar composition to traditional power/liquid type bioceramics. However, they also have thickened agents and several additives such as methylcellulose or glycerin, which help to prevent the cement from hardening in its package. Their additives could alter the biological properties of bioceramics. In the same vein, there was a report that EndoSequence Root Repair Putty showed a statistically significant difference negatively associated with the cell viability of human dermal fibroblasts[23]. In this study, however, CCK-8 using hDPCs revealed that all PPBCs showed biocompatibility comparable to PMTA. Rather, the proliferation tendency was shown depending on time and concentration. A favorable outcome could be expected in the long-term period regardless of the amount of cement.

Mineralization potential is an essential factor as a capping material that covers the exposed pulp and forms reparative dentin functioning as a "biological seal". Tricalcium silicate cement such as MTA has been known to promote osteogenic differentiation and mineralization of human dental pulp via released calcium[35]. EndoSequence cement was the first premixed putty-type cement, and showed similar favorable results in biological evaluations compared with ProRoot MTA[23,36-38]. Fast-set type EndoSequence cement, BCPT used in this study showed the most superior results in ALP and ARS, which was also better than PMTA, and other PPBCs were comparable to PMTA in ALP and ARS staining. In the previous studies using bone marrow stem cells, ECPM showed

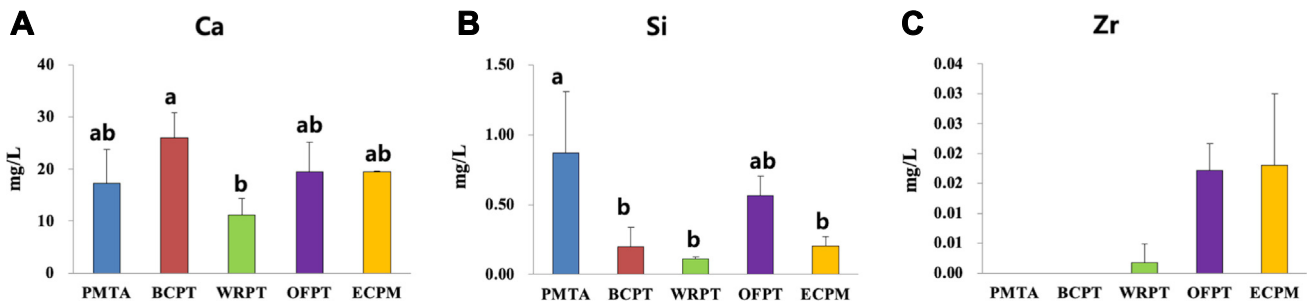


FIGURE 2 – Ion release for 24 hours after 1-hour setting for (*A*) Ca, (*B*) Si, and (*C*) Zr. Each bar represents the average ± standard deviation. Different letters indicate significant differences between groups ($n = 3$, $P < .05$). BCPT, BC RRM fast-set putty; ECPM, Endocem MTA premixed; OFPT, One-Fil PT; PMTA, ProRoot MTA; WRPT, Well-Root PT.

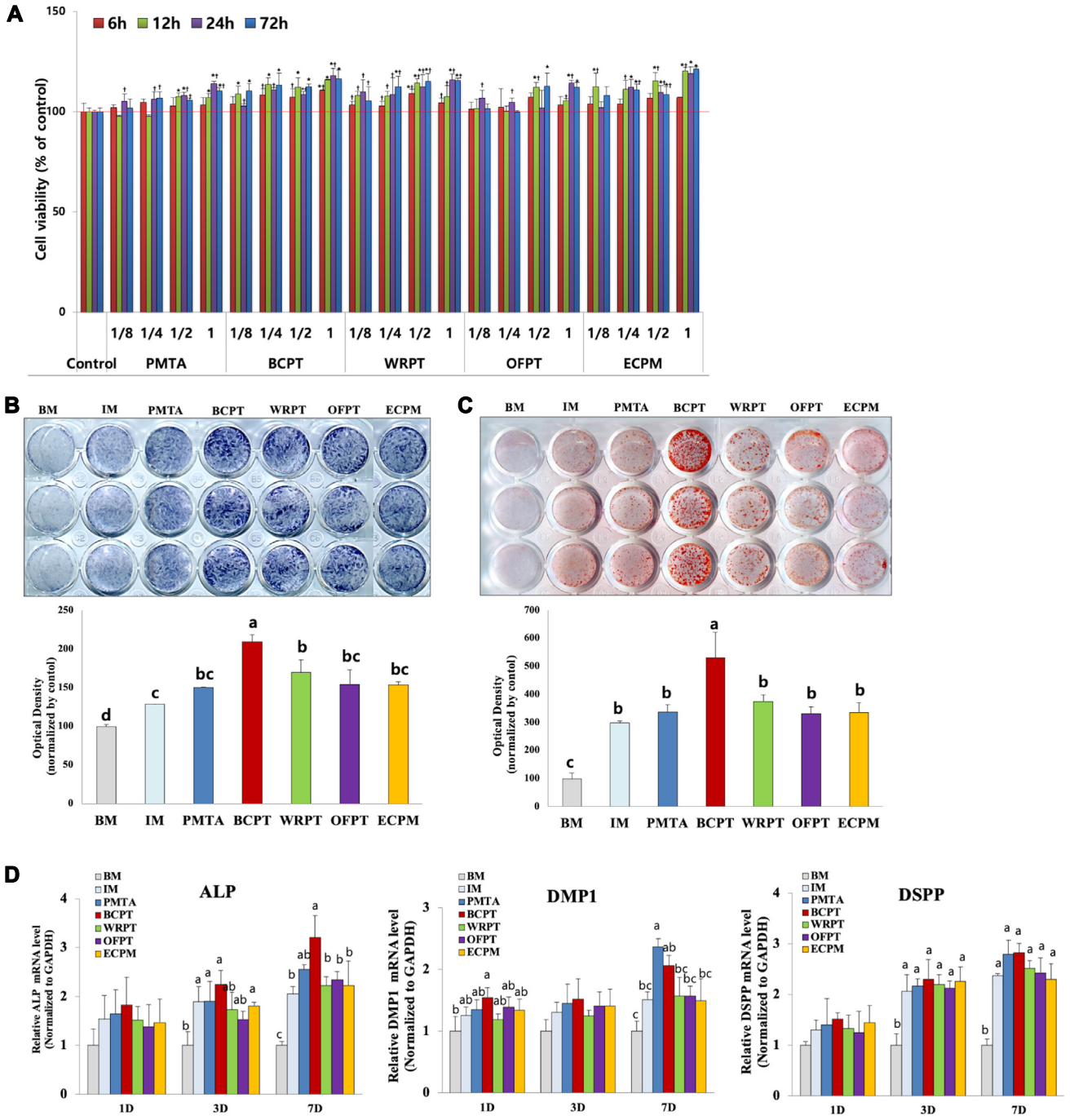


FIGURE 3 – Cytocompatibility and mineralization potentials. (*A*) Cell counting kit-8 (CCK-8) assay for 6, 12, 24, and 72 hours. (*B*) ALP staining and quantification with basal media, induction media, or material extracts (1:2 dilution) revealed alkaline phosphatase (ALP, *purple color*) activity on day 7. (*C*) ARS staining quantification with basal media, induction media, or material extracts (1:2 dilution) revealed calcium deposition on day 14. (*D*) Gene expression for mineralization (ALP, DMP1, and DSPP) from material extracts (1:2 dilution) in hDPCs. Different letters indicate significant differences between groups ($n = 3$, $P < .05$). Each bar represents the mean ± standard deviation. ALP, alkaline phosphatase; ARS, Alzarin Red S; BCPT, BC RRM fast-set putty; BM, basal media; DMP1, dentin matrix acidic phosphoprotein 1; DSPP, dentin sialophosphoprotein; ECPM: Endocem MTA premixed; IM, induction media; OFPT, One-Fil PT; PMTA, ProRoot MTA; WRPT, Well-Root PT.

favorable biocompatibility and osteogenic potential similar to PMTA[38,39], which is consistent with this study using dental pulp cells (DPCs).

In the present study, it was observed that ALP and DSPP markers were not significantly upregulated in DPCs treated with PMTA compared with induction media (Fig. 3*D*). Similar results were also obtained from other PPBCs, which showed outcomes comparable to PMTA. The study revealed that the PPBCs have a mineralization potential comparable to that of PMTA. However, conducting a study designed with a long-term induction period with higher concentration would provide more evident results, consistent with previous studies that demonstrated superior mineralization potential along with

upregulated odontogenic potential markers[40,41].

In clinical usage, PPBCs may offer a significant advantage by providing a homogeneous mixture without the need for a mixing procedure, irrespective of the operator's experience. Furthermore, most PPBCs contain thickening agents, allowing for a variety of viscosities according to the products. Unlike PMTA which has a sandy or mud-like texture and lacks viscosity, PPBCs can be placed with greater stability at the treatment site, especially when dealing with proximal pulpal exposure. In addition, the delivery method, which is provided with a syringe or needle, is also user-friendly and enhances clinical efficiency.

Since the first MTA was introduced, numerous research has been conducted and provided the evidence for using calcium silicate cement in various clinical procedures. To replace traditional cement, PPBCs need sufficient research proving compatibility for various clinical procedures with long-term follow-up checkups.

CONCLUSIONS

PPBCs with similar composition but a different delivery system have several clinical benefits over traditional mixing-type cement. Within the limitation of this study, PPBCs have chemomechanical properties with favorable biocompatibility and mineralization potential, comparable to PMTA. PPBCs would be a suitable replacement for traditional mixing-type cement however, further studies are required to validate these findings and strengthen the conclusions drawn in this study.

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

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