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Original Article

Evaluation of biocompatibility and bioactive potential of Well-Root PT by comparison with ProRoot MTA and Biodentine

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Abstract *Background/purpose:* Well-Root PT is a novel bioceramic material developed to overcome limitations of conventional calcium silicate cements. The purpose of this study was to assess the biocompatibility and bioactivity of a premixed putty-type cement, Well-Root PT.

Materials and methods: Identical cylindrical samples were prepared from ProRoot MTA, Biodentine, and Well-Root PT. *In vitro* calcium weight volume and calcium ion release from the materials were evaluated with scanning electron microscopy and energy-dispersive spectroscopy and inductively coupled plasma-optical emission spectroscopy. An *in vivo* rat direct pulp capping model was implemented with the materials ($n = 14$ per material). The rats were sacrificed at 7 or 28 days. Hematoxylin and eosin and immunohistochemical analyses were performed.

Results: *In vitro* calcium weight volume was $42.83 \pm 8.82\%$ in ProRoot MTA, $47.05 \pm 8.83\%$ in Biodentine, and $29.99 \pm 4.94\%$ in Well-Root PT. Calcium ion releases from Well-Root PT after 7 and 28 days were similar with those from ProRoot MTA, but lower than those from Biodentine ($P = 0.001$ after 7 and 28 days equally). In an *in vivo* rat model, hematoxylin and eosin analysis showed no significant differences in inflammatory infiltration ($P = 0.393$) and hard tissue formation scores among the materials ($P = 0.905$). Also, both CD68 and DSPP expression showed similar results, with no significant differences among the materials (equally $P = 0.874$ for both markers).

Conclusion: Within the limits of this study, Well-Root PT was comparable to ProRoot MTA and Biodentine in terms of biocompatibility and bioactivity.

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Introduction

Dental pulp stem cells (DPSCs) primarily participate in maintaining homeostasis in pulp–dentin complex from the various stimuli. After dental pulp injures, DPSCs can either differentiate into odontoblasts or induce odontoblast-like cell differentiation, thereby promoting hard tissue formation.^{1,2} This process may be affected by a biomaterial for dental pulp.³ Therefore, interaction between pulp tissues and such material would be critical.

In recent years, calcium silicate-based cements have been the standard in the biomaterials for dental pulp due to their biocompatibility and bioactivity.⁴ According to the *in vitro* studies regarding mineral trioxide aggregate (MTA), it promotes odontogenic differentiation and mineralization of DPSCs.^{5–7} In *in vivo* studies, predictable histological outcomes in pulp healing were observed in MTA-treated groups.^{8,9} A previous meta-analysis study reviewed that MTA is less likely to trigger inflammatory response and produces more uniform and dense hard tissue formation than calcium hydroxide.¹⁰ Previous human studies also supported these findings.^{11,12} However, long setting time and tooth discoloration remain the barriers to MTA usage.^{13,14} Biodentine (BD) is a new type of calcium silicate-based cement which shows faster setting and less discoloration than MTA.¹⁵ BD also promotes hard tissue formation by regulating the release of growth factors such as TGF- β 1.^{16,17} However, both MTA and BD require a mixing procedure before the application because they are provided as powder/liquid form. As proper consistency in powder/liquid ratio is difficult to achieve during the mixing procedure,¹⁸ changes in the mechanical or chemical properties of the materials could occur.¹⁹ A previous *in vitro* study found that changes in powder/liquid ratio affects radiopacity, setting time, solubility, and calcium ion release of MTA.²⁰ Another study showed that changes in mixing conditions alter the physical and chemical properties, and microstructure of BD.²¹

Recently, a premixed putty-type bioactive cement, Well-Root PT (WRPT; Vericom Co., Chuncheon, Korea) has been introduced. As WRPT is available in a premixed capsule form,²² it can provide uniform and adequate consistency and clinical convenience.¹⁸ WRPT can be applied directly in the oral cavity by mounting the capsule in a gun. And WRPT has a short setting time than MTA and BD, with an approximately 5 min for initial setting and 45 min for final setting.²³ Despite these advantages, the biocompatibility and bioactivity of WRPT have not been fully studied. Therefore, this study aimed to assess the biocompatibility and bioactivity of WRPT by comparing with MTA and BD in both *in vitro* and *in vivo*. The null hypothesis of this study was that WRPT has a lower priority in terms of pulpal inflammation and hard tissue formation than MTA and BD.

Materials and methods

Sample preparation for *in vitro* analysis

The tested materials were as follows; (i) MTA (ProRoot® MTA; Dentsply Tulsa, OK, USA), (ii) BD (Septodont, Saint Maur de Fossés, France), and (iii) WRPT (WRPT; Vericom Co., Chuncheon, Korea). Identical cylindrical samples (4 mm in diameter and 2 mm in height) from the materials were prepared using a Teflon mold (Washercompany, Gwangmyeong, Korea). MTA and BD were mixed according to the manufacturer's instructions and placed in the mold. WRPT was directly delivered to the mold. The samples were kept in an incubator at 37 °C for 24 h before being removed from the mold.

Assessment of chemical composition

For the assessment of chemical composition of the materials, Fourier transform infrared (FT-IR) spectrometer system (Nicolet5700; Thermo Fisher Scientific, Inc. Waltham, MA, USA) was used. The absorbance of the spectra was measured at wavelengths from 4000 to 400 cm⁻¹ (Fig. 1). The chemical composition was compared based on the peaks in the spectra.

Scanning electron microscopy/energy-dispersive spectroscopy (SEM-EDS) mapping

SEM-EDS mapping was performed to evaluate weight volume (%) of the chemical components on the surface of the materials. Image analysis was performed to the samples ($n = 3$ per group) via FE SEM (High-Resolution FE SEM-III, Hitachi, Japan). The images were obtained at 500 $\times$ and 3000 $\times$ magnifications. The weight volume (%) of the components were calculated based on the peaks in the spectrum.

Inductively coupled plasma-optical emission spectroscopy (ICP-OES) analysis

ICP-OES analysis was performed to measure calcium ion release from the materials. The samples ($n = 5$ per group) were transferred into conical tubes with 10 mL of deionized water and incubated at 37 °C for 28 days. The eluates were collected at 7 and 28 days and analyzed using an ICP-OES machine (ICP-OES 5110, Santa Clara, CA, USA)

In vivo analysis

The *in vivo* study design was reviewed and approved by the Institutional Animal Care and Use Committee of Kyung Hee Medical Center, Seoul, Korea (KHMC-IACUC-2023-013).

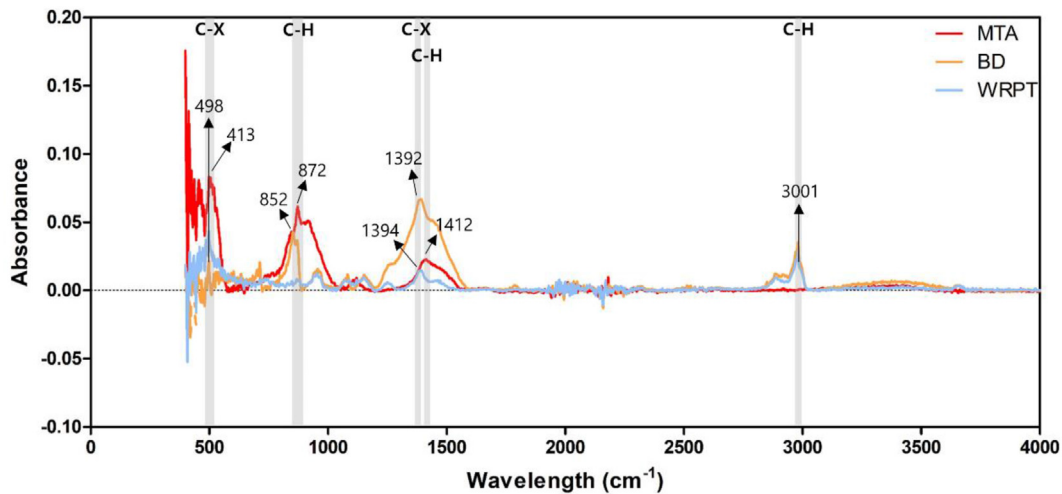


Figure 1 Fourier transform infrared (FT-IR) spectra of the materials. MTA, mineral trioxide aggregate; BD, Biodentine; WRPT, Well-Root PT.

For sample size calculation, G*power 3.1.9.7 software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) was used with a statistical power of 97%, a significance level of 0.05, and an effective sample size of 1.03. Effective sample size was obtained from the primary outcomes of a previous study.²⁴ A minimum of seven samples per group was finally calculated. Following the sample size calculation, the animals were randomly assigned to the materials ($n = 7$ per group).

A rat direct pulp capping model

For the *in vivo* analysis, a rat direct pulp capping model was employed to forty-two 7-week-old male Sprague–Dawley rats weighing 200–300 g. Before the experimental procedure, the animals were given intraperitoneal injection of 30 mg/kg of Zoletil 50 (Virbac Lab, Carros, France) for anesthesia. After intraoral cleaning with cotton pellets immersed in a 2% chlorhexidine solution (Sigma–Aldrich Co., St. Louis, MO, USA), the animals received 2% lidocaine with 1:80,000 epinephrine (YUHAN, Seoul, Korea). Under continuous saline irrigation, pulp exposure was created on the occlusal surface of the left maxillary first molars using a sterile #1/4 round bur. Hemostasis on the exposure site was followed and the materials were then applied. Next, the coronal sealing was performed using a light-curable Light FIX paste (Sun Medical Co., Ltd., Moriyama, Japan). Finally, the animals were given a single dose of penicillin G (20,000 IU) (Alvogen potassium penicillin G, Alvogen, Seoul, Korea). At 7 days after the operation, 21 rats were sacrificed by perfusing 10% neutral buffered formalin solution (Sigma–Aldrich Co.) directly into the bloodstream via aorta under anesthesia with overdose administration of Zoletil 50, and maxillary specimen were collected. And another 21 rats were sacrificed with the same method after 28 days.

Hematoxylin and eosin (H&E) stain analysis

The specimens were fixed in 10% neutral buffered formalin, sectioned along the tooth axis at a thickness of 2–3 μm ,

and attached to glass microscope slides. The sections were then subjected to H&E staining and analyzed using an auto slide scanner (TW-SM01, TaeWoong Medical Co. Ltd, Goyang, Korea). Pulp inflammation was evaluated in the samples sacrificed at day 7, and hard tissue formation was assessed in the specimens sacrificed at day 28. The evaluation was performed using a histological criterion as previously described (Table 1).²⁵ The evaluation was performed by two examiners.

Immunohistochemical (IHC) analysis

To assess biocompatibility and bioactivity, pulp inflammation (CD68) and hard tissue formation (DSPP) markers used in previous studies were selected for IHC staining.²⁶ The samples from the day 7 group were stained with the

Table 1 Evaluation criteria based on the degree of inflammation and hard tissue formation.

Score	Definition
Inflammatory infiltration	
1	Absent or very few inflammatory cells of the ROI
2	Inflammatory cells are observed up to one third of ROI
3	Inflammatory cells are observed up to two thirds of ROI
4	Inflammatory cells are 4 observed more than two thirds of ROI
Hard tissue formation	
1	Hard tissue deposition is observed more than two thirds of ROI or complete dentinal bridge formation is observed
2	Hard tissue deposition is observed up to two third of ROI
3	Hard tissue deposition is observed up to one third of ROI
4	No hard tissue deposition
ROI, region of interest.	

CD68 marker, and the ones from the day 28 group were stained with the DSPP marker. Specific antibodies for CD68 (1:200 dilution, ab-125,212, Abcam, Cambridge, UK) and DSPP (1:500 dilution, LFMB-21, Santa Cruz Biotechnology, Santa Cruz, CA, USA) were used according to the manufacturer's instructions. Expression was then confirmed using 3,3-diaminobenzidine, followed by counterstaining with hematoxylin. The percentage IHC-positive area in the scanned IHC sections was calculated by two examiners using ImageJ software (National Institutes of Health).

Statistical analysis

Data were statistically analyzed using IBM SPSS Statistics 20.0 (IBM Corp., Armonk, NY, USA). To examine inter-observer reliability in H&E and IHC analysis, the intraclass coefficient (ICC) values were calculated and the values ranged from 0.775 to 0.972, indicating highly reliable agreement between the examiners. The results were statistically analyzed with the Kruskal–Wallis test ($P < 0.05$) and the Mann–Whitney U test ($P < 0.017$) as post-hoc analysis.

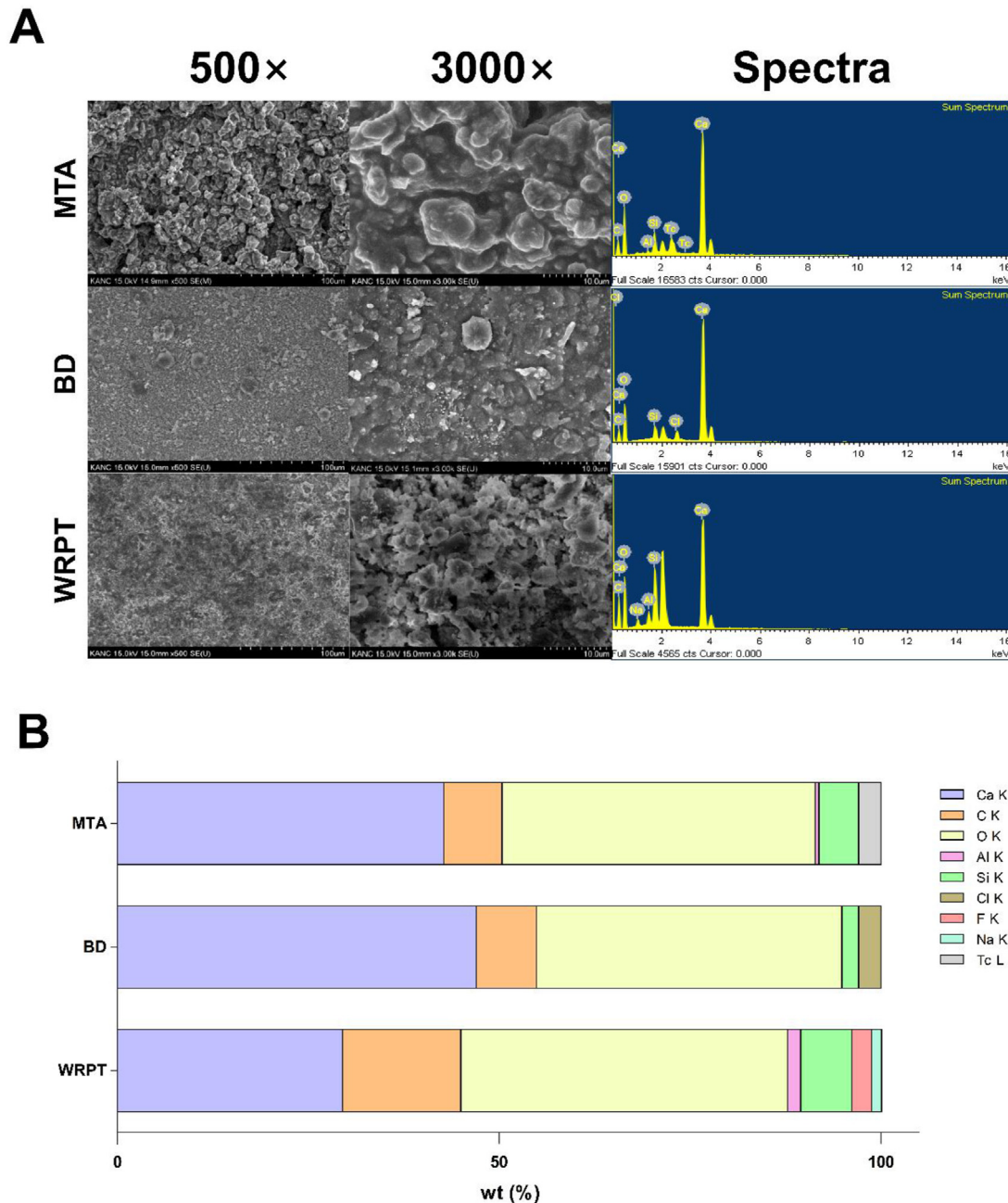


Figure 2 Scanning electron microscopy/energy-dispersive spectroscopy (SEM-EDS) mapping. (A) Representative SEM images with 500 × and 3000 × magnifications and the corresponded EDS spectra. (B) The weight volume (%) of the components. Note that the calcium volume was the highest in BD, followed by MTA and WRPT. MTA, mineral trioxide aggregate; BD, Biodentine; WRPT, Well-Root PT.

Results

Results from *in vitro* study

FT-IR analysis showed that MTA had peaks at 413 cm^{-1} in the C-X group and 1412 and 872 cm^{-1} in the C-H group. BD had peaks in the C-H group (3001 , 852 cm^{-1}) and C-X group (1392 , 498 cm^{-1}). Similarly, WRPT also showed noticeable peak values in the C-H group (3001 cm^{-1}) and C-X group (1394 , 498 cm^{-1}) (Fig. 1). This shows that there are no distinct differences in chemical compositions between the materials. Fig. 2A shows representative SEM images and EDS spectra. The calcium weight volume was $42.83 \pm 8.82\%$ in MTA, $47.05 \pm 8.83\%$ in BD, and $29.99 \pm 4.94\%$ in WRPT (Fig. 2B). Regarding the results of ICP-OES analysis, all materials constantly released calcium ion. The amounts of calcium ion released were $97.74 \pm 16.85\text{ ppm}$ in BD, $33.10 \pm 11.25\text{ ppm}$ in MTA, and $20.87 \pm 8.98\text{ ppm}$ in WRPT at day 7 and $271.91 \pm 92.48\text{ ppm}$ in BD, $124.16 \pm 25.58\text{ ppm}$ in MTA, and $72.37 \pm 8.35\text{ ppm}$ in WRPT at day 28 (Fig. 3). At both measurement times, there was statistical difference

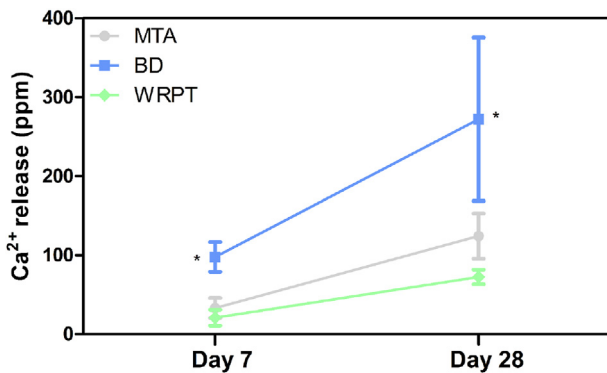


Figure 3 Time-dependent calcium ion release (ppm) from the materials. Asterisk (*) means that there was statistical difference between the material and WRPT ($P < 0.017$). MTA, mineral trioxide aggregate; BD, Biodentine; WRPT, Well-Root PT.

in calcium ion release between BD and WRPT ($P = 0.001$), whereas there was no statistical difference between MTA and WRPT ($P = 0.034$ after 7 days and $P = 0.289$ after 28 days).

Histological and immunohistochemical results

Fig. 4 and Table 2 show the results of H&E stain analysis. There were no significant differences in both inflammatory infiltration ($P = 0.393$) and hard tissue formation scores among the groups ($P = 0.905$). Regarding the IHC analysis, CD68-positive area was $2.4 \pm 0.6\%$ in MTA, $2.5 \pm 2.0\%$ in BD, and $3.4 \pm 2.1\%$ in WRPT on day 7 (Fig. 5A and B). On day 28, DSPP-positive area was $5.2 \pm 3.0\%$ in MTA, $5.2 \pm 1.9\%$ in BD, and $6.1 \pm 1.9\%$ in WRPT (Fig. 5C and D). At both measurement times, there were no significant differences in the staining areas of CD68 and DSPP among the materials ($P = 0.874$ for two markers equally).

Discussion

This study assessed the biocompatibility and bioactivity of WRPT by comparing it with MTA and BD. In this study, *in vitro* results showed that bioactivity of WRPT was comparable to MTA. And *in vivo* results showed that there were no significant differences in biocompatibility and bioactivity among the materials. Therefore, the null hypothesis was rejected.

The material used for vital pulp therapy should have biocompatibility, potential to promote formation of hard tissues such as reparative dentin, antibacterial property, and inducing immune reaction.^{27,28} MTA has demonstrated favorable treatment outcomes and has been a standard material for vital pulp therapy since its development, fulfilling the requirements of materials for vital pulp therapy. This material has been widely used in vital pulp therapy including a direct or indirect capping, regenerative endodontic procedures and pulpotomy.^{29,30} This was found to be suitable for use as a pulp capping agent in animal studies.^{26,31} Previous studies on WRPT reported that the

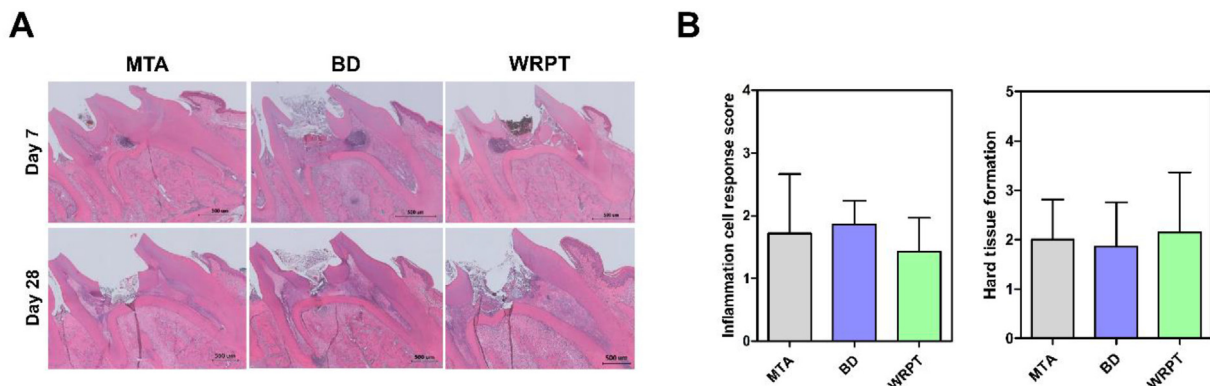


Figure 4 Hematoxylin and eosin (H&E) analysis. (A) Representative histological images. (B) Bar graphs of inflammation cell response score and hard tissue formation. Note that there were no significant differences in the histologic scores among the materials ($P = 0.393$ in inflammation cell response score and $P = 0.905$ in hard tissue formation, P -values from Kruskal–Wallis test). MTA, mineral trioxide aggregate; BD, Biodentine; WRPT, Well-Root PT.

Table 2 Numerical values of histological analysis.

Grade	Inflammatory cell response score				Hard tissue formation			
	1	2	3	4	1	2	3	4
MTA	4/7	1/7	2/7	0/7	2/7	3/7	2/7	0/7
BD	1/7	6/7	0/7	0/7	3/7	2/7	2/7	0/7
WRPT	4/7	3/7	0/7	0/7	3/7	1/7	2/7	1/7

MTA, mineral trioxide aggregate; BD, Biodentine; WRPT, Well-Root PT.

material also met the requirements of vital pulp therapy material.^{18,32} Similar to MTA, it has been reported that WRPT exhibits antibacterial properties by generating an alkaline environment after application and that it releases calcium ions to form odontoblasts or odontoblast-like cells which produce hard tissue via pulp cell differentiation.³²

Regarding biocompatibility of WRPT, there were no differences in CD68-positive area among three pulp capping materials. And this result was consistent with the results of

inflammation evaluation by H&E staining. Several studies have already shown that MTA and BD cause similar pulp inflammation.^{33,34} Therefore, it is suggested that WRPT, which causes similar mild inflammation, can be used as an alternative material to MTA and BD. CD68 is abundantly expressed by circulating and tissue macrophages, and monocyte lineage cells including osteoclasts.^{35,36} As the cells positive for this marker play a crucial role in inflammatory response such as phagocytosis and innate immune reaction, CD68 marker can describe the inflammation in a specific tissue sample.³⁶

The results of this study showed that WRPT has a bioactive potential comparable to MTA and BD. WRPT showed comparable results to MTA and BD in hard tissue formation. Also, the same results were observed in DSPP expression. The higher the release of calcium ions, the more osteoblasts differentiate and produce a higher pH, which promotes hard tissue formation.³⁷ According to the *in vitro* results of this study, BD was found to have a significantly higher concentration of released calcium ions compared to the others, which is consistent with previous studies on

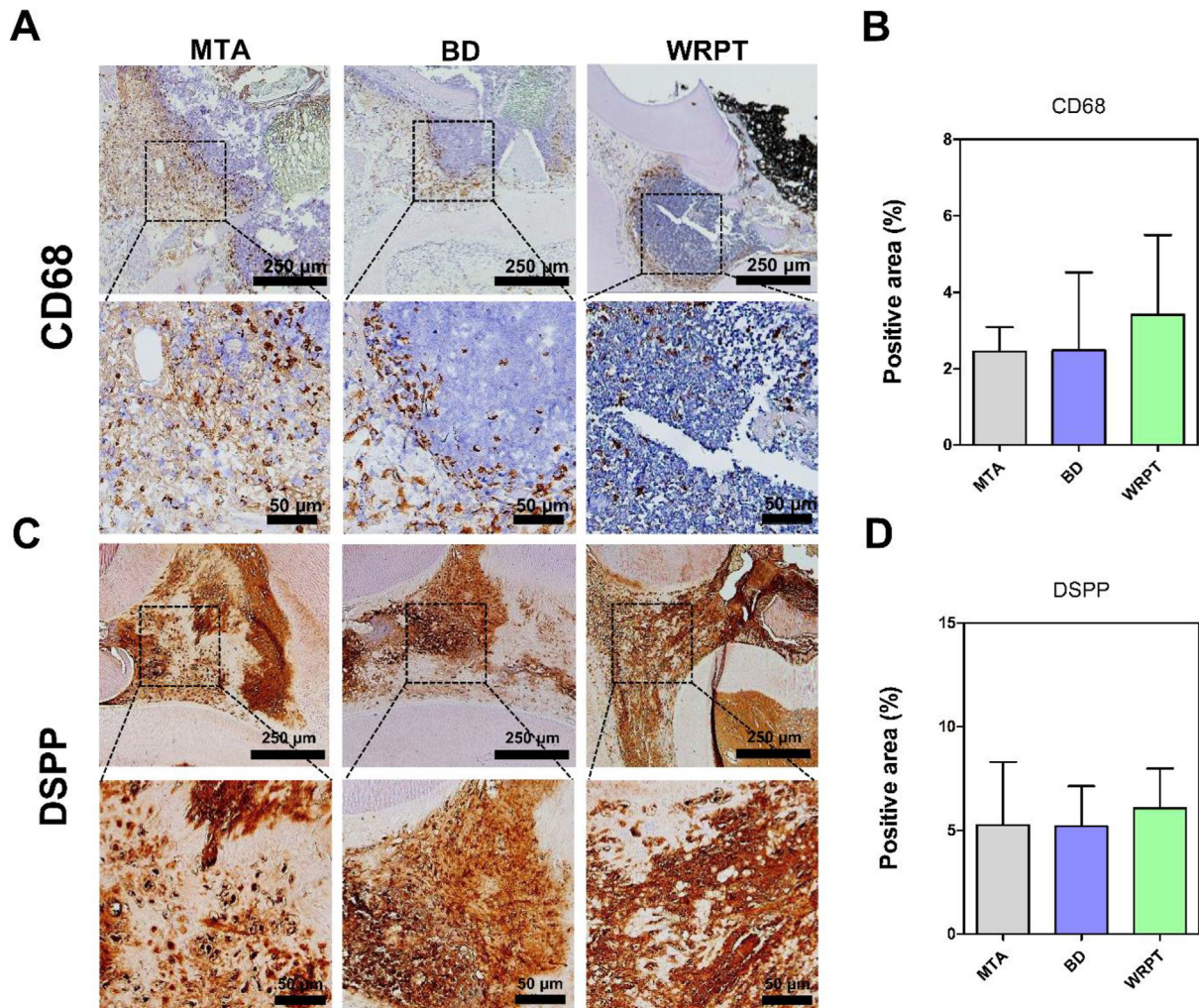


Figure 5 Immunohistochemical (IHC) analysis. (A) Representative CD68-stained images. (B) Bar graph of CD68-positive area (%). A low degree of CD68 expression was observed in all materials with no statistical difference. (C) Representative DSPP-stained images. (D) Bar graph of DSPP-positive area (%). Note that no significant differences in DSPP expression among the materials. MTA, mineral trioxide aggregate; BD, Biodentine; WRPT, Well-Root PT.

BD.^{38,39} Despite MTA showed a lower calcium ion release concentration than BD, MTA was reported to produce a sufficient amount of hard tissue and showed favorable treatment outcomes in several clinical studies.⁴⁰ This has also been demonstrated in animal study, with no difference in the degree of hard tissue formation between MTA and BD.³⁴ Therefore, the release of calcium ions by MTA can be clinically considered sufficient to form hard tissue after direct pulp capping. Likewise, though the concentration of calcium ion release from WRPT was significantly lower than that of BD, the bioactivity of WRPT can be considered similar to that of the two materials used in this experiment, considering the results of hard tissue formation assessment and IHC evaluation using DSPP marker.

Furthermore, because of its product form, WRPT has superior properties to the two materials. The physical and chemical characteristics of the materials may differ based on the powder/liquid ratio since MTA and BD need to be mixed immediately before usage. The changes in physical and chemical properties of bioceramics could cause longer setting time, higher solubility, lower mechanical hardness and lower calcium ion release.^{20,21} As a result of this, these materials may be washed out or their ability to induce hard tissue formation may be reduced and ultimately this change could negatively affect the outcome of the direct pulp capping.³⁰ Whereas, because WRPT is a premixed type material, it has a uniform composition even in the mixed state, which has the advantage of highly predictable treatment results.¹⁸ For the same reason, it also has the advantage of lower technical sensitivity concerning mixing and shorter treatment time. It also has superior operability over the other two materials as it is a premixed paste packed in a capsule, allowing the required amount to be applied to the appropriate point and this makes lower clinician-sensitivity.

However, there were limitations of this study. Due to the small number of samples, non-parametric statistics were used. As a result, as differences between each material had significance, but direct comparison of average values was not possible. In addition to this, as the inflammatory response was evaluated only after 7 days, chronic inflammation could not be assessed. Future study with larger sample number and longer period of observation is needed.

In conclusion, Well-Root PT is a premixed putty-type bioactive cement with clinical convenience for vital pulp therapy. Within the limits of this study, the biocompatibility and bioactivity of Well-Root PT is compatible with ProRoot MTA and Biodentine.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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