Development of a novel fluorapatite-forming calcium phosphate cement with calcium silicate: *In vitro* and *in vivo* characteristics

Yusuke SUZUKI¹, Makoto HAYASHI¹, Takuya YASUKAWA¹, Hiroshi KOBAYASHI¹, Kosuke MAKINO¹, Yoriyuki HIRANO¹, Shozo TAKAGI², Laurence C. CHOW² and Bunnai OGISO¹

¹ Department of Endodontics, Nihon University School of Dentistry, 1-8-13 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-8310, Japan

² American Dental Association Foundation, Dr. Anthony Volpe Research Center, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA

Corresponding author, Makoto HAYASHI; E-mail: hayashi.makoto53@nihon-u.ac.jp

Aim of this study was to develop a novel fluorapatite-forming calcium phosphate cement (FA-CPC) with tricalcium silicate (TCS) for endodontic applications and to examine its *in vitro* and *in vivo* characteristics. The FA-CPC powder consisted of 62.8% CaHPO₄, 30.8% CaCO₃, and 6.4% NaF. One part of TCS was combined with 9 parts of FA-CPC powder (FA-CPC with TCS). A 1.5 M phosphate solution was used as cement liquid. Setting time (ST), diametral tensile strength (DTS), phase composition by X-ray diffraction (XRD), and cement alkalinity were analyzed. Cement biocompatibility was assessed using rat subcutaneous model. Cement ST was 10.3±0.6 min and DTS was 3.89±0.76 MPa. XRD patterns showed that highly crystalline apatitic material was the only significant phase present and pH value was approximate 11.0. FA-CPC with TCS demonstrated similar biocompatibility as that of mineral trioxide aggregate control. These results suggest that FA-CPC with TCS may be useful for endodontic applications.

Keywords: Calcium phosphate cement, Fluorapatite, Calcium silicate, Endodontic cement

INTRODUCTION

Major endodontic failures occur as a result of leakage of irritants from pathologically involved root canals into the periradicular tissues¹. Ideal orthograde and/or retrograde filling materials should seal off the pathways of communication between the root canal system and its surrounding periodontal tissues². Additionally, these materials should be biocompatible with the host tissues, insoluble in tissue fluids, and easily manipulated³. Although many types of endodontic materials, such as gutta-percha, zinc oxide eugenol-based cement, composite resin, and glass ionomer cement, have been introduced over the years, most exhibit different levels of weakness. Mineral trioxide aggregate (MTA) materials, developed in 1993 by Torabinejad *et al.*³, overcame most of these weaknesses and have been in widespread use of endodontic treatments. However, an undesirable property of this material was its difficult handling properties, namely, relatively long setting times⁴. Thus, it is needed to develop even more ideal materials for endodontic treatments.

Calcium phosphate cement (CPC) that initially contains an equimolar mixture of tetracalcium phosphate (TTCP), Ca₄(PO₄)₂O and dicalcium phosphate anhydrous (DCPA), CaHPO₄ has self-hardening characteristics and forms hydroxyapatite (HA) as the end product⁵. The CPC has become a subject of interest in dental and medical applications as bone graft biomaterials, due to its setting property, biocompatibility, and osteoconductivity^{6,7}. More importantly, it is gradually replaced by new bone without volume loss⁸. It is important to note that CPC of different compositions can form different end

products in addition to HA, such as dicalcium phosphate dihydrate⁹, octacalcium phosphate⁹, or fluorapatite (FA)¹⁰. Because the end products of CPC may control resorption rates, CPC can be developed to have resorption rates suitable for diverse clinical applications.

Takagi *et al.* developed self-hardening CPCs based on DCPA and CaO or CaCO₃¹¹. A preliminary study indicated that the addition of sodium fluoride (NaF) to these CPCs also formed fluoridated HA or FA and CaF₂¹².

For endodontic treatments such as root-end filling, perforation repair, apexification, pulp-capping, or pulpotomy, it is desirable to have CPC that is biocompatible and osteoconductive, yet non-bioresorbable in soft and hard tissue. Because dissolution in a cell-mediated acidic environment leads to *in vivo* resorption, CPC that forms end products that have little or practically no solubility in such acidic conditions can be predicted to be essentially non-resorbable. It is well described in the literature that fully or partially fluoridated HA materials have significantly lower solubility in acids¹³ and also promote bone formation in the rat tibia and dog mandible^{14,15}. Additionally, Cherng *et al.* showed that the addition of tricalcium silicate (TCS) improved the sealing ability and raised the pH of CPC to levels comparable to that produced by MTA¹⁶. Hence, FA-forming CPC with TCS can be expected to have much lower resorbability than HA-forming CPC, good sealing ability, and sufficient alkalinity. The purpose of this study was to develop a novel FA-forming CPC with TCS for endodontic applications and to examine its physiochemical properties and biocompatibility.

MATERIALS AND METHODS

Test materials

All chemicals used in this study were reagent grade purchased from J.T. Baker Chemical Co. (NJ, USA). FA-forming CPC powder without TCS consisted of 62.8% (% denotes mass percent) DCPA (CaHPO_4), 30.8% calcium carbonate (CaCO_3), and 6.4% sodium fluoride (NaF), as described previously¹⁷. A batch of DCPA with a median particle size of $6.5 \pm 0.3 \mu\text{m}$ ($n=3$) was produced by grinding DCPA in a blender (model 38BL52 LBC10, Waring Commercial, Torrington, CT, USA). CaCO_3 was ground in a planetary ball mill (Retsch PM4, Brinkman, NY, USA) in cyclohexane for 24 h to produce a median particle size of $2.6 \pm 0.4 \mu\text{m}$ ($n=3$). TCS was prepared by combining 3 moles of CaO with 1 mole of fumed silica (SiO_2) in water and stirred for 8 h. The slurry was filtered, dried, and then heated at $1,450^\circ\text{C}$. The resulting powder was ground to a median size of $20 \mu\text{m}$. To prepare FA-forming CPC powder with TCS, one part of TCS was added to 9 parts of FA-forming CPC powder. A 1.5 M sodium phosphate solution (pH 5.6) was used as the cement liquid. MTA (ProRoot MTA, Dentsply Tulsa, TN, USA) was used according to manufacturers' instructions as the control.

Physicochemical analysis

1. Setting time (ST) and diametral tensile strength (DTS) measurements

All samples for ST and DTS measurements were prepared by mixing cement powder and liquid at the powder (g)/liquid (mL) ratio (P/L) of 3 to produce a cohesive paste. For the ST measurement, the paste was packed into a stainless steel mold (6-mm diameter $\times$ 3-mm height) sandwiched between two glass plates, and stored in a humidor with 100% relative humidity at 37°C . The ST was measured using the Gillmore needle method (ADA specification #9). The test was performed using a needle with a tip diameter of 1.06 mm loaded with 453.5 g of weight. In this method, the cement was considered to have set when the needle with the load failed to make a perceptible indentation on the surface. The ST was the average time obtained from 3 specimens.

For the DTS measurement, specimens were prepared as described previously¹⁸. The paste was packed into a stainless-steel mold, formed by two rods (diameter: 6 mm) in a cylindrical cavity, with 2.8 MPa of applied pressure; the mold was placed in an incubator at 37°C and 100% humidity for 4 h. The specimens were then removed from the molds, and individually immersed in 10 mL of a physiological-like solution (PLS)¹⁹, containing 1.15 mM Ca, 1.2 mM P, 133 mM NaCl, and 50 mM HEPES buffer, with the pH adjusted to 7.4, at 37°C for 20 h. After immersion in PLS, the diameter and length of each specimen were measured with a micrometer, and the sample was placed on a universal testing machine (United Calibration Corp, Garden Grove, CA, USA) for the DTS measurement. The specimen was stressed between steel platens that were covered with one thickness of wet filter paper and crushed at a loading

rate of 10 mm/min. The force at failure was recorded and converted into a stress in MPa unit. The DTS value presents the average value obtained from 5 specimens.

2. X-ray diffraction (XRD) and cement alkalinity analyses
The phases present in the FA-forming CPC with TCS were determined by powder XRD analysis. After the DTS measurement, the fractured specimens were dehydrated in 100% ethanol for 1 h and dried in a desiccator for 3 days. The dried specimens were ground into a fine powder with a mortar and pestle, and characterized by XRD. The XRD patterns of the specimens were recorded using a vertically-mounted diffractometer system (D/MAX 2000, Rigaku, Danvers, MA, USA), with graphite-monochromatized CuK_α radiation ($\lambda=0.1540 \text{ nm}$) generated at 40 kV and 40 mA. The sample was scanned from 10 to 50 degrees 2θ in continuous mode ($2^\circ 2\theta \text{ min}^{-1}$, time constant 2 s). The unreacted powder of FA-forming CPC with TCS was also assessed using the same XRD procedure.

The alkalinity of FA-forming CPC with or without TCS, and that of MTA was measured using a pH meter (pH 500 series, Cole-Parmer, Vernon Hills, IL, USA). The pH values of a 1 mL aliquot of 30 mM KCl solution after the addition of 0.25 g of pulverized set FA-forming CPC with or without TCS or set MTA were measured for 10 min to evaluate cement alkalinity. The set sample was ground by a mortar and pestle, and 0.25 g of the sample was placed in 1 mL of 30 mM KCl solution. A flat-end combination pH electrode (Thomas Scientific, model #S450CBNC, Swedesboro, NJ, USA) was connected to the pH meter, and the pH was measured continuously for 10 min. The sample suspension was under constant stirring (350 rpm) and the pH of the solution was monitored. The standard uncertainty of the pH measurement was estimated to be 0.01 pH units.

Biocompatibility analysis

The biocompatibility of FA-forming CPC with TCS in the subcutaneous tissue of rats was examined. This study was permitted by and performed under guidelines specified by the Animal Experimentation Committee at Nihon University School of Dentistry, with experiments carried out at the Nihon University School of Dentistry animal laboratory.

1. Animal preparation and surgical procedure

Thirty 7-week-old male Donryu rats, weighing 200–250 g, were divided into three test periods (7, 21, or 42 days; $n=10$ in each experimental period). Material implantation was performed using procedures modified from a previously method¹⁰. Briefly, each animal was anesthetized by intraperitoneal injection of pentobarbital sodium (Kyoritsu Seiyaku Co., Tokyo, Japan) at a dose of 50 mg/kg body weight. Under general anesthesia, the back area of the rat was shaved and swabbed with 70% volume fraction ethanol. Subcutaneous pockets were created to receive test materials in the dorsal region of the rat. Horizontal incisions approximately 15 mm in length were made

along each side of the back bone, and subcutaneous skin pockets were created by blunt dissection. To prevent interactions among the materials being tested, the tubes were placed at least 20 mm apart.

FA-forming CPC with TCS was prepared for biocompatibility analysis according to a previously described method²⁰, meanwhile, MTA was prepared according to the manufacturers' instructions. Sterile polyethylene tubes (length: 8 mm; inner diameter: 0.8 mm) with one end closed were divided into 3 groups: negative control group (empty polyethylene tubes), an MTA group (polyethylene tube filled with MTA), and an FA-forming CPC with TCS group (polyethylene tube filled with FA-forming CPC with TCS). After mixing of these cements, these were immediately filled into the tubes. Each of these tubes was inserted into a pocket of subcutaneous tissues, and then the pocket was closed with interrupted sutures.

2. Tissue preparation and histological evaluation

Each group of animals was euthanized by anesthesia overdose at the end of each time period. The implants and surrounding tissues were carefully removed and fixed in 10% neutral formalin for 2 weeks, and the specimens were embedded in paraffin. Tissue sections were made longitudinally through the middle of the tubes and 3 sections from each specimen were selected randomly. These sections were subjected to hematoxylin and eosin (H-E) staining for histopathological evaluation.

Histopathological evaluation was performed in microscopic fields adjacent to experimental materials at the open ends of the tubes under a light microscope (DFC500, Leica Microsystems, Wetzlar, Germany) at $\times 100$, $\times 200$, and $\times 400$ magnifications by an observer that was blind to the procedure. Evaluation of the inflammatory reaction was conducted according to Cox and Robbin's criteria^{21,22} focused on the accumulation of acute and chronic inflammatory cells, fibrin deposits, tissue edema, and vascular congestion. These criteria were classified into 4 grades: Grade I, scattered and wavy collagen fiber deposits and fibrosis; Grade II, infiltration of inflammatory cells, and wavy collagen fiber deposits and fibrosis; Grade III, dense infiltration of inflammatory cells, limited areas of tissue edema and vascular congestion; and Grade IV, very dense infiltration of acute and chronic inflammatory cells, widespread edematous areas and vascular congestion along with fibrin deposits.

Statistical analysis

Statistical analysis on ST and DTS values of FA-forming CPC with TCS and FA-forming CPC without TCS was performed using Student's *t*-test. The significance of the differences in the severity of inflammation was determined using two-way ANOVA and Tukey's test. Statistical significance was defined as $p < 0.05$.

RESULTS

Physicochemical analysis

1. ST and DTS measurements

The ST (mean $\pm$ SD: $n=3$) of FA-forming CPC with and without TCS was 10.3 ± 0.6 min and 14.0 ± 1.0 min, respectively. The DTS (mean $\pm$ SD: $n=5$) of FA-forming CPC with and without TCS was 3.89 ± 0.76 MPa and 2.65 ± 0.29 MPa, respectively (Table 1). ST decreased with the addition of TCS while, in contrast, DTS increased with the addition of TCS. A significant difference was detected between these cements in both properties.

2. XRD and cement alkalinity analyses

The powder XRD pattern of FA-forming CPC with TCS showed that highly crystalline apatitic material was the main phase in the hardened cement (Fig. 1). The pH values of FA-forming CPC with and without

Fig. 1 XRD patterns of FA-forming CPC with TCS before and after setting. FA: fluorapatite, D: DCPA, C: CaCO_3 , TCS: tricalcium silicate

Table 1 Setting time (ST) and diametral tensile strength (DTS) of fluorapatite (FA)-forming calcium phosphate cement (CPC) with or without tricalcium silicate (TCS)

	ST (min)	DTS (MPa)
FA-forming CPC with TCS	10.3 ± 0.6^a	3.89 ± 0.76^a
FA-forming CPC without TCS	14.0 ± 1.0^b	2.65 ± 0.29^b

Mean $\pm$ SD

In each analysis, values with a different lower-case letter are significantly different ($p < 0.05$).

TCS, and that of MTA all increased rapidly after the addition of 0.25 g of pulverized set cements and became nearly constant at approximately 9.0, 11.0 and 13.0, respectively (Fig. 2).

Biocompatibility analysis

1. Period of 7 days (Figs.3 a, b, and c)

The inflammatory response among all groups was similar in period of 7 days. The main characteristics are the presence of infiltration of inflammatory cells with collagen fiber deposits and limited areas of vascular congestion. In the FA-forming CPC with TCS group, 3.3, 46.7, and 50.0% of the specimens indicated Grade

Fig. 2 The pH response curves of FA-forming CPC with and without TCS, and MTA.

I, Grade II, and Grade III inflammation, respectively. In the MTA group, 3.3, 40.0, and 56.7% of the specimens had Grade I, Grade II, and Grade III inflammation, respectively. In the control group, 6.7, 60.0, and 33.3% of the specimen revealed Grade I, Grade II, and Grade III inflammation, respectively.

2. Period of 21 days

The inflammatory response for all groups decreased with time. A thick fibrous capsule formation around open-end of the polyethylene tube was observed. In the FA-forming CPC with TCS group, 66.7, 23.3, and 10.0% of the specimens demonstrated Grade I, Grade II, and Grade III inflammation, respectively. In the MTA group, 33.3, 63.3, and 3.3% of the specimen exhibited Grade I, Grade II, and Grade III inflammation, respectively. In the control group, 60.0, 36.7, and 3.3% of the specimens indicated Grade I, Grade II, and Grade III inflammation, respectively.

3. Period of 42 days (Figs. 3 d, e, and f)

The tissue response of three groups was very similar to the 21 days period. In the FA-forming CPC with TCS group, 86.7 and 13.3% of the specimens indicated Grade I and Grade II inflammation, respectively. In the MTA group, 83.3 and 16.7% of the specimens had Grade I and Grade II inflammation, respectively. In the control group, 90.0 and 10.0% of the specimens revealed Grade I and Grade II inflammation, respectively.

4. Comparison of inflammatory response

Means of inflammation grades in histopathological evaluations of all experimental groups are indicated

Fig. 3 Histopathological findings of the tissue response at the open ends of polyethylene tubes (asterisks).

At 7 and 41 days: the control group (a and d), FA-forming CPC with TCS group (b and e), and MTA group (c and f). Arrow: infiltration of inflammatory cells; open arrow: wavy collagen fiber deposits and fibrosis; arrowhead: vascular congestion; open arrowheads: thin fibrous capsule formations at the open ends of polyethylene tubes without infiltration of inflammatory cells (scale bars=50 μ m).

Fig. 4 Comparison of inflammation response in three groups at each test period.

The inflammation grades in histopathological assessment were determined according to Cox and Robbin's criterion. Each value represents mean $\pm$ SD ($n=10$). Values connected by a horizontal line are significantly different (** $p<0.01$).

in Fig. 4. Within the same experimental period, no-significant difference was detected among the groups. In contrast, the mean inflammation grade for the period of 7 days was significantly higher than those for 21 days and 42 days in the same group ($p<0.01$). No-significant difference was observed between 21 and 42 days for control and FA-forming with TCS groups ($p>0.05$), however, the MTA group exhibited a significant difference ($p<0.01$).

DISCUSSION

MTA has been used widely in endodontic treatments, due to not only its superior sealing ability but also its biocompatibility. Nevertheless, one of the main drawbacks of MTA is its extended setting period and prolonged maturation phase. The longer setting time of MTA, in comparison with Portland cement, is attributed to the lower levels of sulfur and tricalcium aluminate in MTA²³. Although many have attempted to shorten the setting time of MTA by various accelerators to overcome this clinical disadvantage^{24,25}, adding various elements can adversely affect the ideal characteristics of MTA⁴. Therefore, we developed FA-forming CPC with TCS as a novel endodontic cement. The ST of FA-forming CPC with TCS (10.3 $\pm$ 0.6 min) was significantly shorter than that of the cement without TCS (14 $\pm$ 1.0 min). Additionally, the ST of FA-forming CPC with TCS is much shorter than the initial setting time of MTA using the Gillmore needle method according to ASTM C266, which was reported to 45 $\pm$ 2.9 min²⁶. When used for various endodontic treatments, this difference may be of important clinical significance.

The DTS value of FA-forming CPC with TCS was significantly higher than that without TCS as determined in the present study. Huang *et al.*²⁶ reported a DTS value of 4.4 $\pm$ 0.1 MPa for a 24-h specimen of MTA, which is marginally higher than the DTS (3.89 $\pm$ 0.76 MPa) of FA-Forming CPC with TCS. Endodontic materials do not bear direct pressure during function, hence, a high DTS

value is not believed to be as an important requirement as for materials used to repair or restore defects in load-bearing sites. Thus, FA-forming CPC with TCS should be sufficient for most endodontic applications and was focused in the powder XRD pattern analysis.

The powder of FA-forming CPC with TCS used in this study contained NaF as a source of fluoride. When mixed with phosphate solution, the powder XRD pattern (Fig. 1) revealed that highly crystalline apatitic material was the only significant phase present. It is known that FA typically has very high crystallinity compared to HA, and also TCS was added to the FA-forming CPC¹², final product of the FA-forming CPC with TCS was FA. FA has the more stable characteristic than HA because FA is significantly less soluble than HA or tooth mineral itself under neutral and acidic pH conditions¹³. Therefore, FA-forming CPC with TCS may be suitable for endodontic applications that require the material to have high stability for a long time. Although Takagi *et al.*¹⁰ described that a CPC that contains TTCP and DCPA mixed with an H₃PO₄ solution including hydrogen fluoride could also form FA, the cement that uses NaF as a source of fluoride in this study may be clinically desirable from a safety viewpoint, because of the hazardous nature of hydrogen fluoride.

Our results showed that higher alkalinity of FA-forming CPC with TCS compared to that without TCS. This was attributed to the highly alkaline TCS compound. The pH value of MTA was higher than that of FA-forming CPC with TCS. Calcium hydroxide, an important endodontic materials, is generally effective at eradicating intraradicular bacteria due to its alkaline pH²⁷. Tronstad *et al.*²⁸ demonstrated that the pH values of dentin adjacent to calcium hydroxide change their pH range to 11.0–12.2 after calcium hydroxide is placed in the root canals. Because the pH value of FA-forming CPC with TCS is similar to that of calcium hydroxide, FA-forming CPC with TCS may maintain the local state of alkalinity necessary for bone or dentin formation.

Because the characteristics of ST, DTS, and cement alkalinity for endodontic applications of FA-forming CPC with TCS is better than those of FA-forming CPC without TCS, the biocompatibility analyses of FA-forming CPC with TCS and MTA were analyzed. These analyses used in this study involved subcutaneous implant methods, in which the materials to be evaluated were placed in polyethylene tubes that were implanted in the subcutaneous connective tissue in rats. This method was introduced by Torneck²⁹ and has become one of the most common methods for the evaluation of dental-material biocompatibility. The placement of the material to be evaluated in the terminal portions of the polyethylene tubes prevented the diffusion of the material into the connective tissue; thus, this method is preferred to direct injection into the subcutaneous connective tissue³⁰. Several inflammatory assessment methods of tissue response adjacent to materials have been used in rat connective tissue. This study used Cox and Robin's criterion, which is based on the accumulation of acute and chronic inflammatory cells, fibrin deposits,

tissue edema, and vascular congestion. This innovative method for assessment is considered to be more precise for comparison of the severity of the inflammation around the tubes containing the materials, due to the attention to vascular and reparative response³¹⁾.

In the biocompatibility analysis, the inflammatory response decreased over time in all three groups. The mean inflammation grade in each group at 7 days was significantly higher than those at 21 and 42 days. At the same time no-significant differences were observed among the three groups for each of the three time periods. This demonstrates that neither FA-forming CPC with TCS nor MTA stimulated the surrounding tissues more than the negative control group (empty tube). After 21 days, the mean inflammation grade decreased significantly in each group, with no-significant difference among the three groups. Additionally, no-significant difference was evident between 21 and 42 days for the control and FA-forming CPC with TCS groups; however, the MTA group exhibited a significant difference. Consequently, the severity of the inflammatory responses adjacent to FA-forming CPC with TCS and MTA were identical, and equal to that of the negative control group during this experimental period. Thus, it is thought that FA-forming CPC with TCS presented the same tissue response and biocompatibility as MTA, which is known for its excellent biocompatibility, when tested in rat subcutaneous connective tissue.

According to the findings of this study, it is concluded that the setting time of FA-forming CPC with TCS is shorter than that of MTA and it can form FA and presents adequate DTS, pH value, and biocompatibility. Therefore, FA-forming CPC with TCS may be useful for endodontic applications. Nevertheless, further studies on the sealing ability and biological properties of this cement are required before its use in clinical endodontic applications.

ACKNOWLEDGMENTS

We would like to thank Dr. Masatake ASANO for his contribution to this research effort. This research work was financially supported in part by a Grant-in-Aid for Scientific Research (C-#26462898) from the Japan Society for the Promotion of Science (M.H.) and grant from the Dental Research Center, Nihon University School of Dentistry for 2012, 2013, and 2014.

REFERENCES

- 1) Siqueira JF Jr., Rocas IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. *J Endod* 2008; 34: 1291-1301.
- 2) Chng HK, Islam I, Yap AU, Tong YW, Koh ET. Properties of a new root-end filling material. *J Endod* 2005; 31: 665-668.
- 3) Torabinejad M, Watson TF, Pitt Ford TR. Sealing ability of a mineral trioxide aggregate when used as a root end filling material. *J Endod* 1993; 19: 591-595.
- 4) Parioikh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature review Part III: Clinical applications, drawbacks, and mechanism of action. *J Endod* 2010; 36: 400-413.
- 5) Brown WE, Chow LC. A new calcium phosphate, water-setting cement. In: Brown PW, editor. *Cement Research Progress*. Westerville, OH: American ceramic society; 1986. p. 352-379.
- 6) Sugawara A, Fujikawa K, Takagi S, Chow LC. Histological analysis of calcium phosphate bone grafts for surgically created periodontal bone defects in dogs. *Dent Mater J* 2008; 27: 787-794.
- 7) Sugawara A, Nishiyama M, Kusama K, Moro I, Nishimura S, Kudo I, Chow LC, Takagi S. Histopathological reactions of calcium phosphate cement. *Dent Mater J* 1992; 11: 11-16.
- 8) Mirtchi AA, Lemaitre J, Terao N. Calcium phosphate cements: study of the beta-tricalcium phosphate-monocalcium phosphate system. *Biomaterials* 1989; 10: 475-480.
- 9) Markovic M, Chow LC. An octacalcium phosphate forming cement. *J Res Natl Inst Stand Technol* 2010; 115: 257-265.
- 10) Takagi S, Frukhtbeyn S, Chow LC, Sugawara A, Fujikawa K, Ogata H, Hayashi M, Ogiso B. In vitro and in vivo characteristics of fluorapatite-forming calcium phosphate cements. *J Res Natl Inst Stand Technol* 2010; 115: 267-276.
- 11) Takagi S, Chow LC, Ishikawa K. Formation of hydroxyapatite in new calcium phosphate cements. *Biomaterials* 1998; 19: 1593-1599.
- 12) Takagi S, Ogata H, Chow LC. Properties of DCPA+CaO and DCPA+CaCO₃ fluorapatite-forming calcium phosphate cements (abstract 2476). *J Dent Res (Spec Iss B)* 2011; 90.
- 13) Chow LC, Markovic M. Physicochemical properties of fluorapatite. In: Amjad Z, editor. *Calcium phosphates in biological and industrial systems*. Norwell, MA: Kluwer Academic Pub; 1998. p. 67-84.
- 14) Inoue M, Rodriguez AP, Nagai N, Nagatsuka H, LeGeros RZ, Tsujigiwa H, Inoue M, Kishimoto E, Takagi S. Effect of fluoride-substituted apatite on in vivo bone formation. *J Biomater Appl* 2011; 25: 811-824.
- 15) Sakae T, Ookubo A, LeGeros RZ, Shimogoryou R, Sato Y, Lin S, Yamamoto H, Kozawa Y. Bone formation induced by several carbonate and fluoride containing apatite implanted in dog mandible. *Key Eng Mater* 2003; 240: 395-398.
- 16) Chong AM, Takagi S, Chow LC. Acid neutralization capacity of a tricalcium silicate-containing phosphate cement as an endodontic materials. *J Res Natl Inst Stand Technol* 2010; 115: 471-476.
- 17) Chow LC, Takagi S, Wang W, Frukhtbeyn S. A new fluorapatite and calcium silicate cement for endodontic treatments (abstract 482). *J Dent Res (Spec Iss A)* 2013; 92.
- 18) Chow LC, Hirayama S, Takagi S, Parry E. Diametral tensile strength and compressive strength of a calcium phosphate cement: effect of applied pressure. *J Biomed Mater Res* 2000; 53: 511-517.
- 19) Takagi S, Chow LC, Hirayama S, Sugawara A. Premixed calcium-phosphate cement pastes. *J Biomed Mater Res B Appl Biomater* 2003; 67: 689-696.
- 20) Suzuki Y, Hayashi M, Ogata H, Yasukawa T, Takagi S, Chow LC, Ogiso B. Biocompatibility of fluorapatite cement with calcium silicate for endodontic treatment (abstract 123). *J Dent Res (Spec Iss B)* 2013; 92.
- 21) Cox CF, Subay RK, Suzuki S, Suzuki SH, Ostro E. Biocompatibility of various dental materials: pulp healing with a surface seal. *Int J Periodontics Restorative Dent* 1996; 16: 240-251.
- 22) Robbins S, Cortan R, Kumar V. *Pocket compaction to Robbins pathologic basis of diseases*. 6th ed. Philadelphia, PA: WB Saunders; 1991. p.45-46.
- 23) Dammaschke T, Gerth HU, Zuchner H, Schafer E. Chemical and physical surface and bulk material characterization of white ProRoot MTA and two Portland cements. *Dent Mater* 2005; 21: 731-738.
- 24) Kogan P, He J, Glickman GN, Watanabe I. The effects of

- various additives on setting properties of MTA. *J Endod* 2006; 32: 569-572.
- 25) Wiltbank KB, Schwartz SA, Schindler WG. Effect of selected accelerants on the physical properties of mineral trioxide aggregate and Portland cement. *J Endod* 2007; 33: 1235-1238.
- 26) Huang TH, Shie MY, Kao CT, Ding SJ. The effect of setting accelerator on properties of mineral trioxide aggregate. *J Endod* 2008; 34: 590-593.
- 27) Pawinska M, Skrzydlewska E. Release of hydroxyl ions from calcium hydroxide preparations used in endodontic treatment. *Rocz Akad Med Bialymst* 2003; 48: 145-149.
- 28) Tronstad L, Andreasen JO, Hasselgren G, Kristerson L, Riis I. pH changes in dental tissues after root canal filling with calcium hydroxide. *J Endod* 1981; 7: 17-21.
- 29) Torneck CD. Reaction of rat connective tissue to polyethylene tube implants. I. *Oral Surg Oral Med Oral Pathol* 1966; 21: 379-387.
- 30) Olsson B, Sliwowski A, Langeland K. Subcutaneous implantation for the biological evaluation of endodontic materials. *J Endod* 1981; 7: 355-367.
- 31) Shahi S, Rahimi S, Lotfi M, Yavari H, Gaderian A. A comparative study of the biocompatibility of three root-end filling materials in rat connective tissue. *J Endod* 2006; 32: 776-780.