RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2009.35.358

ScienceAsia 35 (2009): **358–364**

Investigation of the mechanical and in vitro biological properties of ordinary and white Portland cements

Pincha Torkittikul, Arnon Chaipanich*

Cement and Concrete Research Laboratory, Department of Physics and Material Science, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

*Corresponding author, e-mail: arnon@chiangmai.ac.th

Received 20 Nov 2008
Accepted 13 Oct 2009

ABSTRACT: Biomaterials containing calcium silicate have been widely used in dental and bone repaired applications. This study investigated mechanical and in vitro biological properties of ordinary and white Portland cement both of which are composed mainly of calcium silicates. The time of setting was determined using a Vicat needle. Compressive strength was measured at the age of 1, 3, and 7 days. The 1 day set Portland cement pastes were soaked in a simulated body fluid to investigate the ability of the material to form a bond with living bone tissue. X-ray diffraction traces of samples following immersion in simulated body fluid demonstrated that both cement pastes form a hydroxyapatite layer within 7 days but white Portland cement shows a noticeably higher amount of apatite crystal than that of ordinary Portland cement. Scanning electron micrographs reveal a clear presence of hydroxyapatite on the surface of white, but not in ordinary Portland cement. In addition, white Portland cement can achieve a compressive strength of 30 MPa within 24 h after curing in distilled water at human temperature.

KEYWORDS: hydroxyapatite, bioactivity, setting time, compressive strength

INTRODUCTION

When the body exhibits disease or damage, defence mechanisms will stimulate the formation of a fibrous capsule around an artificial implant material in an attempt to isolate it from surrounding tissue. Glass and ceramic materials have been shown to form stable bonds with living bone tissue in vitro [1–4]. In 1969, the ability of material to form a bond with living bone tissue, referred to as bioactivity, was first observed [1]. Materials that exhibit bioactivity can therefore be used to repair damaged bone tissue.

Silicates have been shown to accelerate the formation of new bone tissue by inducing genetic activity of bone-regulating cells [2–7]. When a silicate is immersed in human plasma environment, a precipitation of ions forms. As human plasma is supersaturated with respect to hydroxyapatite, Si-OH functional groups on the material surface induce the nucleation of apatite crystals that grow spontaneously. The structure of the hydroxyapatite layer formation is similar to the mineral component of bone and provides a focus for the attachment and proliferation of new bone-forming cells [8–10].

Kokubo et al [10] developed a cellular simulated body fluid (SBF) which contains ion concentrations similar to those of human plasma in order to reproduce formation of apatite on bioactive materials in vitro. The hydroxyapatite layer formation on the material surface within 4 weeks of exposure to SBF in vitro gives an indication that the material is bioactive and will form a bond with living tissue.

A range of calcium silicate based materials are currently being investigated for their potential use in dental and bone-contact applications [11–14]. Previous research [15] has demonstrated the in vitro bioactivity of the calcium sulphate hemihydrate-tricalcium silicate composite bone cement. Huan and Chang [15] reported that silicate species could play an important role in forming hydroxyapatite in the simulated body environment and the formation of a homogeneous apatite layer on the material surface was dependent on the presence of tricalcium silicate.

For the past decade, mineral trioxide aggregate (MTA), consisting of 80% wt white Portland cement and 20% wt bismuth oxide, has been used as a root repair material in dentistry [16–22]. It was found that MTA shows bioactivity and clinical success in the sealing of connections between the root canal system and the surrounding tissues. In spite of the increasing potential clinical applications, the bioactivity of cement based material in SBF is barely known. Accordingly, the bioactivity of ordinary Portland cement and white Portland cement in vitro was carried out in SBF in

order to determine whether they form hydroxyapatite. The pH values of the SBF solutions containing both cements was also measured. In the addition, the setting and mechanical properties of both types of Portland cement were investigated.

MATERIALS AND METHODS

Preparation and characterization

Ordinary Portland cement (OPC) and white Portland cement (WPC) used in this investigation were produced by Siam Cement Public Company Limited. Cement paste samples were prepared in a polypropylene beaker with sterile distilled water at a water to cement ratio of 0.5 by mass. In each case, 50 g of cement was manually blended with 25 g of sterile distilled water using a polypropylene spatula. The specimens were then cast into a rubber mould (10 mm in diameter and 2 mm in height), sealed with plastic wrap, and cured at 23 ± 2 °C for 24 h. After curing, the sample were removed from the rubber moulds and their chemical compositions were determined at the Electron Microscopy Research and Service Centre of Chiang Mai University using low vacuum SEM with a JEOL JEM-5910LV microscope linked to an energy dispersive X-ray analyser (EDX).

Setting time of the OPC and WPC paste

The time of setting of Portland cement paste was determined using a Vicat needle according to ASTM C191-99[23]. The apparatus consisted of a movable rod (300 g, 10 mm in diameter) and a steel needle (1 mm diameter, 50 mm length). Portland cement powder (650 g) was mixed with the amount of distilled water required for normal consistency[24]. After mixing, a ring mould filled with the cement sample was placed on a base plate and the excess paste at the top of the mould was removed. A steel needle was plunged into the Portland cement paste every 10 min. The initial setting time is determined as the time necessary to achieve a penetration of 25 mm and the final setting time as the total time when the needle does not sink into the paste.

Compressive strength of the OPC and WPC paste

The compressive strength of the test materials was determined according to ASTM D695-91[25]. Portland cement powder was mixed with water using a constant water to cement ratio (W/C) of 0.5, and the mixture was cast in a cylindrical mould (6 mm diameter $\times$ 10 mm high). After setting for 24 h, the specimens were removed from the moulds and were cured in a water bath at 37 °C. The compressive strengths of the samples were measured at 1, 3, and 7 days using Hounsfield test equipment (H10KS-0407) at a loading rate of 0.5 mm/min. The reported results are the averages of five specimens.

Bioactivity in vitro and dissolution

Simulated body fluid (SBF) was prepared according to the procedure described by Kokubo[10]. The ion concentrations of the SBF solution are similar to those in human blood plasma. OPC and WPC paste disks at 1 day (10 mm in diameter and 2 mm in height) were immersed in the SBF solution in hermetically sealed polypropylene containers under sessile conditions at 37 °C for 7 days with a surface area-to-volume ratio of 0.1 cm^{-1} [26]. The pH values of SBF liquid were measured at 1, 3, and 7 days using an electrolyte-type pH meter (Ringer Ω Metrom 713 pH Meter). After 7 days, the disc specimens were removed from the SBF solution, gently rinsed with deionized water, and dried in air at room temperature. The surface morphologies and crystalline phase of the OPC and WPC paste discs after immersion in SBF were determined by SEM (JEOL JEM-5910LV) and X-ray diffraction (Philips PW-1729).

RESULTS

Characterisation of OPC and WPC before immersing in SBF

SEM characterization of the surface of OPC and WPC paste after setting for 1 day has demonstrated that the surfaces of the samples, taken as-cast from the mould before immersing in SBF, were smooth (Fig. 1). An EDX spectrum of OPC and WPC surface confirms the presence of the main elemental constituents which are calcium, silicon, aluminium, and oxygen (Fig. 2).

Setting time and compressive strength of Portland cement paste

It can be seen that the setting time of WPC was noticeably faster than that of OPC (Fig. 3). The results show that the initial and final setting times of OPC were 134 and 190 min respectively and that of WPC were 130 and 170 min respectively.

The compressive strength of WPC and OPC pastes after storage in a 100% humidity water bath at 37 °C for 1, 3, and 7 days is shown in Fig. 4. The result indicated that the compressive strength of Portland cement paste increased with increasing curing period and there was no significant difference between OPC and WPC paste specimens. In the addition, after 7 days of curing in distilled water at human body temperature, OPC and WPC paste specimens achieved

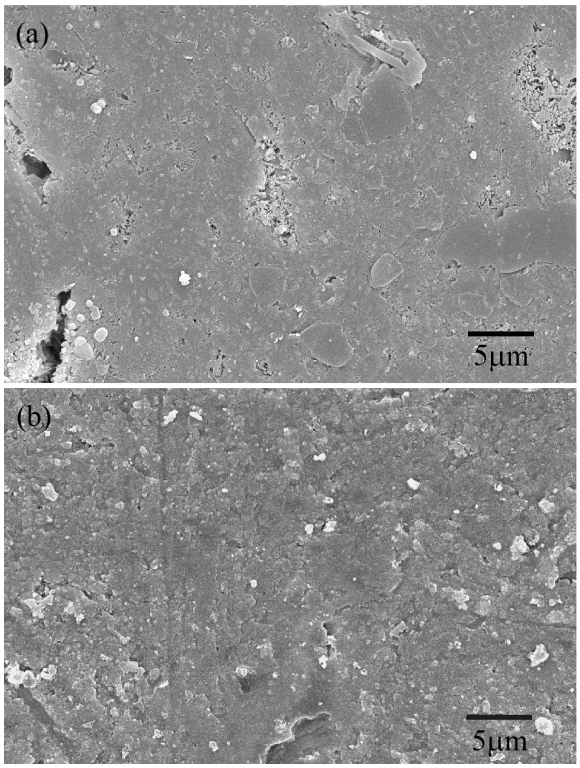

Fig. 1 SEM micrographs of the surface of (a) OPC paste disc and (b) WPC paste disc after setting for 1 day.

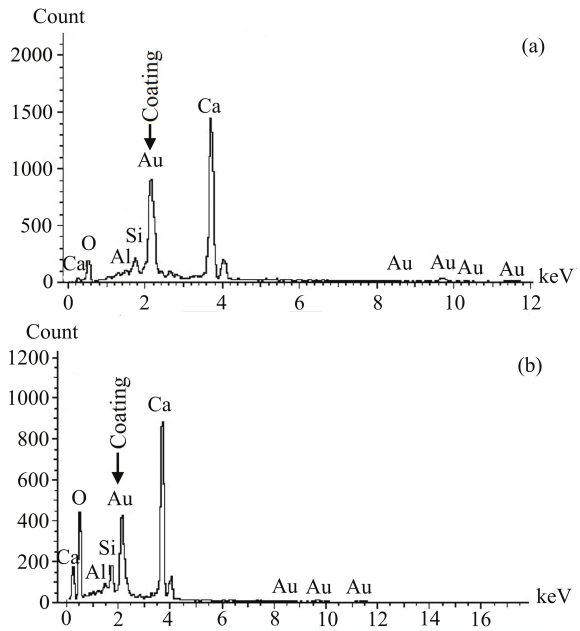

Fig. 2 EDX spectrum of (a) OPC paste disc surface (b) WPC paste disc surface after setting for 1 day.

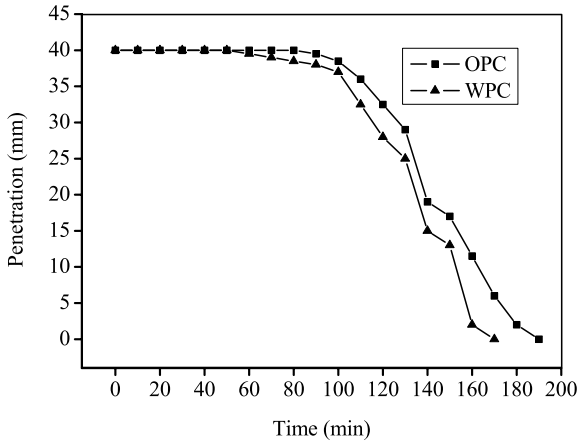

Fig. 3 The initial and final setting times of OPC and WPC paste samples.

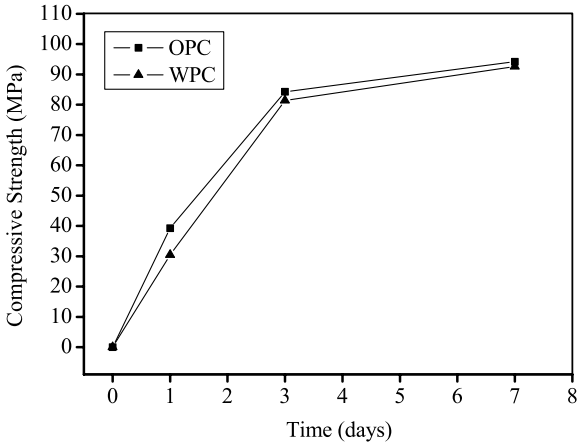

Fig. 4 The compressive strengths of OPC and WPC paste specimens at various times.

compressive strength of 94.2 MPa and 92.6 MPa respectively.

Dissolution characteristics

After 1 week of contact with an OPC paste disc with a surface area-to-volume ratio of 0.1 cm^{-1}, the pH of SBF increased from 7.25 to 9.25 (Fig. 5). In the case of WPC the pH of SBF increased to 9.06.

Bioactivity in vitro

The SEM analyses of the paste disc surfaces following immersion in SBF solution for 7 days are shown in Fig. 6. Formation of hydroxyapatite was only observed on the WPC surface. The surface of WPC after reaction with SBF is seen to support a particle deposit of similar morphology to the apatite layer reported to

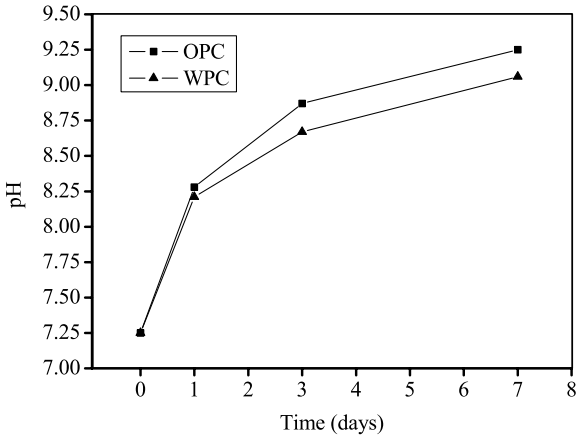

Fig. 5 pH of simulated body fluid after 7 days of exposure to OPC and WPC paste discs.

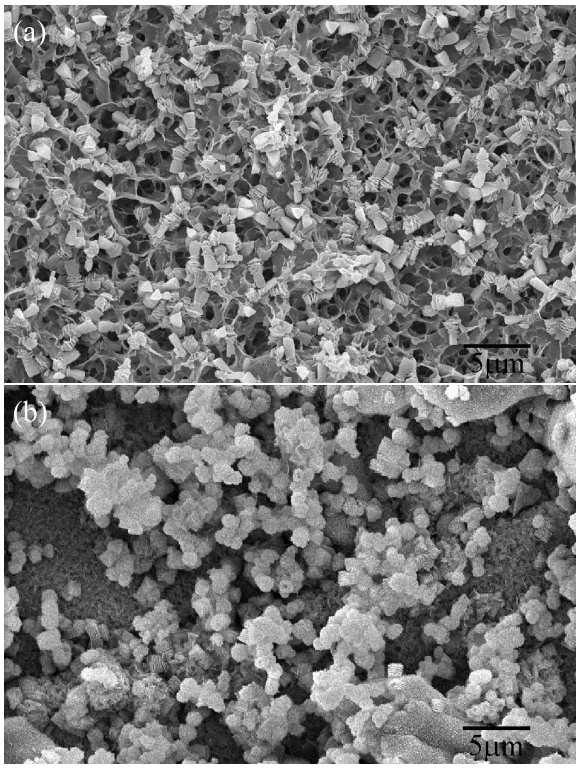

Fig. 6 SEM micrographs of the surface of (a) OPC paste disc and (b) WPC paste disc following immersion in SBF solution for 7 days.

form on bioactive material[27–29]. The higher magnification SEM micrograph shows that small particles of an apatite formed as agglomerates (Fig. 7). An EDX spectrum of the surface of OPC and WPC paste following a residence time of 7 days in SBF is shown in Fig. 8. The spectrum demonstrates that phosphorus

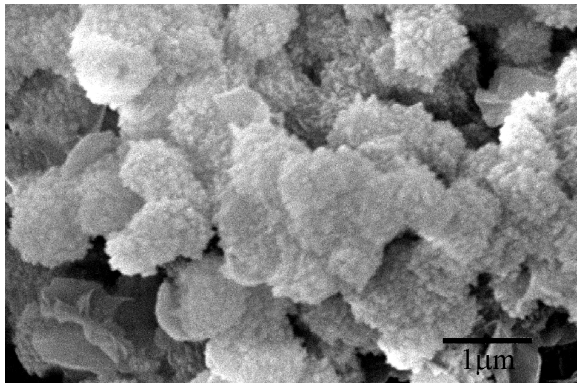

Fig. 7 High magnification SEM micrograph of hydroxyapatite on WPC paste surface.

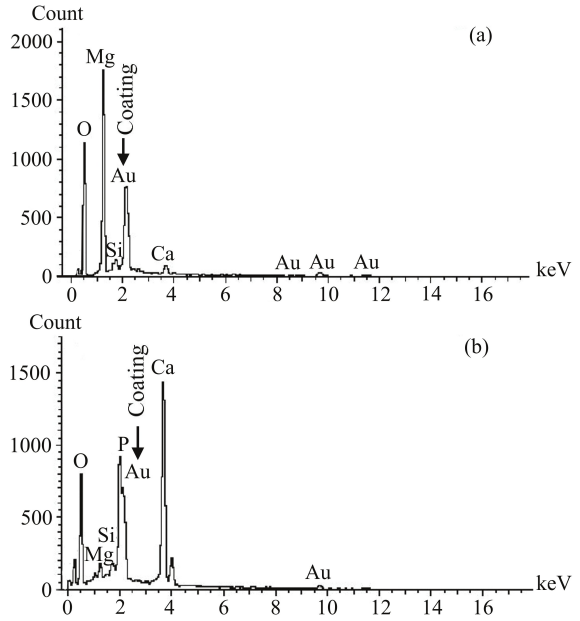

Fig. 8 EDX spectrum of (a) OPC (b) WPC paste disc surfaces following immersion in SBF solution for 7 days.

was present in the WPC paste after immersion but this was not observed for the OPC paste. Elemental mapping also shows that WPC surface had a high concentration of P (Fig. 9). These results confirm the formation of an apatite layer on the surface of WPC paste disc after immersion in SBF solution[30].

The X-ray diffraction (XRD) of the samples after immersion in SBF is shown in Fig. 10. The XRD pattern of OPC and WPC paste discs showed characteristic peaks of hydroxyapatite at $2\theta = 25.9$, 31.8, 32.9, and 34.1. On account of the larger peak for WPC at 31.8, it can be inferred that WPC induces a larger amount of apatite crystal.

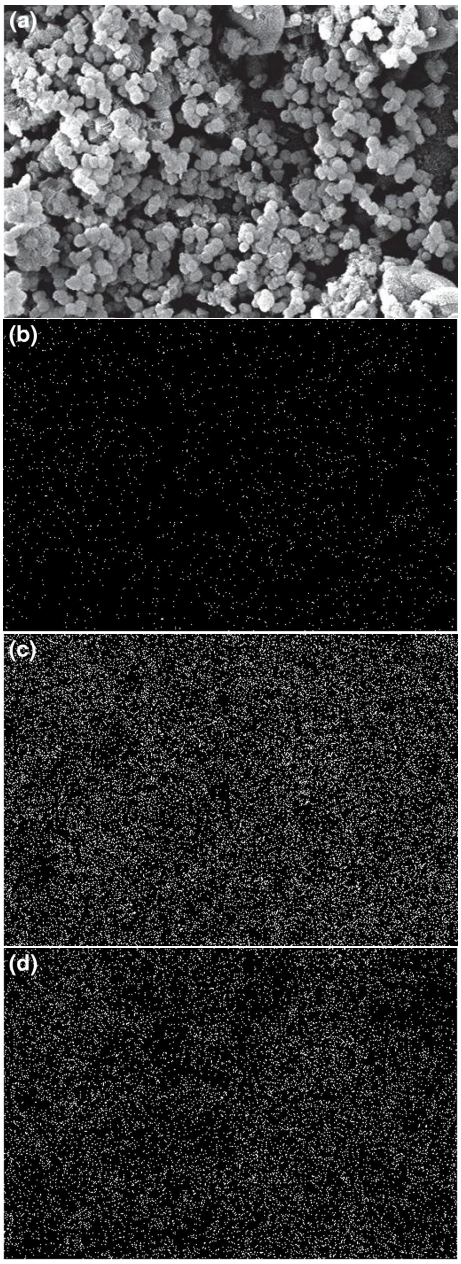

Fig. 9 (a) Hydroxyapatite crystals precipitated on WPC paste surface; distribution of (b) silicon (c) calcium (d) phosphorous.

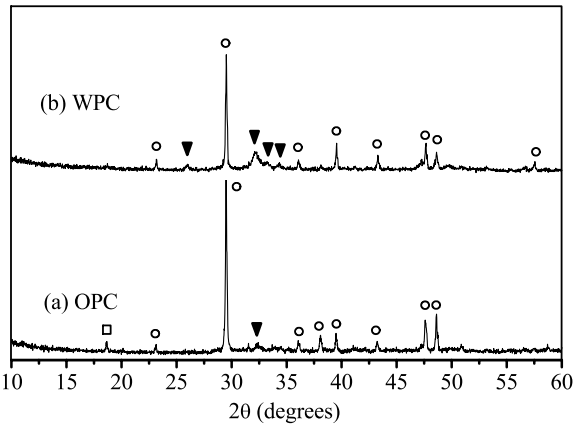

Fig. 10 XRD data for paste disc surface of (a) OPC (b) WPC after immersion in SBF for 7 days. □ calcium hydroxide; ○ calcium carbonate; ▼ hydroxyapatite.

DISCUSSION

Calcium phosphate cement has been successfully used in clinics as bone repair biomaterial for many years[31–36]. However, poor mechanical properties limit any further applications[37–39]. In this study, both OPC and WPC pastes can achieve a compressive strength more than 25 MPa within 24 h after curing in a water bath at 37 °C. With a high initial strength, OPC and WPC may be beneficial for clinical applications that require a certain high initial strength in the first stage of implantation. Portland cement mainly consists of tricalcium silicates, C_3S, and dicalcium silicates, C_2S, which react with water during the hydration[40].

On hydrating the cement, calcium silicate hydrate (CSH) and calcium hydroxide are produced. These provide strength and alkalinity to the material, respectively[40]. As time proceeds, the CSH phase formation increases giving a denser and more homogeneous cement matrix. This results in the formation of a solid network which affords a moderate increase in the mechanical strength and densification. The decrease in the initially high porosity is due to the formation of hydration products. In this study, when Portland paste disc was immersed in SBF solution, calcium hydroxide is leached from the Portland cement matrix. The released OH^- ions are partially buffered and cause a moderate increase in pH from 7.25 to approximately 9.

Bioactivity is shown by the formation of a hydroxyapatite layer on the surface of a material after immersion in SBF solution[3]. The Si-OH functional groups on the surface of silicate material, such as silicate glasses and ceramics, have been shown to act as nucleation centres for hydroxyapatite precipitation[3,41,42]. Recent research has shown that an increase in the super saturation of Ca^+ is not sufficient to promote the formation of new crystals of hydroxyapatite on the material surface in the SBF environment[43,44] and the formation of this homogeneous hydroxyapatite layer is dependent on the content of silicate material[15]. This explains why OPC shows lower intensity peak of

hydroxyapatite than WPC. When OPC is immersed in SBF, silicate ions are likely to concentrate around the CSH gel, and thus the formation of apatite crystal is limited in the CSH[15]. This may be due to the lower proportion of silicate species in OPC compared to WPC. Hence, it is thought that the deposition of hydroxyapatite layer onto the Portland cement paste surface is attributed to both the dissolution of calcium hydroxide and to the high proportion of preexisting Si-OH nucleation sites presented by the nanoporous calcium silicate hydrate gel structure. However, evidence of apatite formation on the OPC surface was not observed in the SEM micrograph. This may be because a very low amount of apatite cannot be detected by an SEM. Accordingly, the results of the XRD, SEM, and EDX analysis show that WPC paste, following immersion in SBF solution, can induce hydroxyapatite precipitation on the material surface within 7 days. This finding confirms the established bioactivity of the WPC paste.

Our study has indicated that the likely mechanism of bonding between WPC paste and viable bone tissue is the spontaneous formation of an intermediate layer of hydroxyapatite on contact with human plasma. However, as a material containing a large amount of calcium hydroxide, which is known to produce a pH of around 12.4 when in contact with water, the hydrated cement paste may induce cytotoxicity[45]. Such a result has been found in previous studies on biocompatibility and cytotoxicity of calcium hydroxide based root canal sealers[46,47]. Further research is now warranted to establish the biocompatibility and cytotoxicity properties to confirm the stimulatory effect of WPC for use in orthopaedic surgery.

Acknowledgements: Financial support from the Thailand Research Fund through the Royal Golden Jubilee PhD Program (Grant No. PHD/0281/2550) is acknowledged. The authors also gratefully acknowledged the Thailand Research Fund for Research Career Development Grant to the second author. The Graduate School of Chiang Mai University is acknowledged for additional support.

REFERENCES

1. Hench LL, Anderson Ö (1993) Bioactive glasses. In: Hench LL, Wilson J (eds) *An Introduction to Bioceramics*, World Scientific, Singapore, pp 41–6.
2. Hence LL, West JK (1996) Biological applications of bioactive glasses. *Life Chem Rep* **13**, 187–241.
3. Kokubo T (1992) Bioactivity of bioglasses and glass ceramics. In: Ducheyne P, Kokubo T, Blitterswijk CA (eds) *Bone-bonding Biomaterials*, Leidendorp, Reed Healthcare Communications, pp 31–46.
4. Hench LL (1997) Sol-gel materials for bioceramic application. *Curr Opin Solid State Mater Sci* **2**, 604–10.
5. Portera AE, Patela N, Skepperb JN, Besta SM, Bonfielda W (2003) Comparison of in vivo dissolution processes in hydroxyapatite and silicon-substituted hydroxyapatite bioceramics. *Biomaterials* **24**, 4609–20.
6. Cao W, Hench LL (1996) Bioactive Materials. *Ceram Int* **22**, 493–507.
7. Hench LL (1998) Biomaterials: a forecast for the future. *Biomaterials* **19**, 1419–23.
8. Kotani S, Fujita Y, Kitsugi T, Nakamura T, Yamamuro T (1991) Bone bonding mechanism of β-tricalcium phosphate. *J Biomed Mater Res* **25**, 1303–15.
9. Fujita Y, Yamamuro T, Nakamura T, Kotani S (1991) The bonding behaviour of calcite to bone. *J Biomed Mater Res* **25**, 991–1003.
10. Kokubo T, Takadama H (2006) How to useful is SBF in predicting in vivo bone bioactivity? *Biomaterials* **27**, 2907–15.
11. Ribeiro DA, Duarte MH, Matsumoto MA, Marques MA, Salvadori DF (2006) Biocompatibility In Vitro Tests of Mineral Trioxide Aggregate and Regular and White Portland Cements. *J Endod* **31**, 605–7.
12. Giovanna GM, Silvia F, David P, Gasparotto G, Carlo P (2008) Calcium silicate coating derived from Portland cement as treatment for hypersensitive dentine. *J Dent* **36**, 565–78.
13. Saidon J, He J, Zhu Q, Safavi K, Spångberg LSW (2003) Cell and tissue reactions to mineral trioxide aggregate and Portland cement. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **95**, 483–9.
14. D, Abdullah Pitt Ford TR, Papaioannou S, J, Nicholson McOnald F (2002) An evaluation of accelerated Portland cement as a restorative material. *Biomaterials* **23**, 4001–10.
15. Huan Z, Chang J (2007) Self-setting properties and in vitro bioactivity of calcium sulfate hemihydrate-tricalcium silicate composite bone cements. *Acta Biomaterialia* **3**, 952–60.
16. Holland R, de Souza V, Nery MJ, Filho JAO, Bernabe PFE, Dezan E (1999) Reaction of dogs' teeth to root canal filling with mineral trioxide aggregate or a glass ionomer sealer. *J Endod* **25**, 728–30.
17. Hachmeister DR, Schindler WG, Walker WA, Thomas DD (2002) The sealing ability and retention characteristics of mineral trioxide aggregate in a model of apexification. *J Endod* **28**, 386–90.
18. Saidon J, He J, Zhu Q, Safavi K, Spångberg LSW (2003) Cell and tissue reactions to mineral trioxide aggregate and Portland cement. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **95**, 483–9.
19. Valois CRA, Costa ED (2004) Influence of the thickness of mineral trioxide aggregate on sealing ability of root-end fillings in vitro. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **97**, 108–11.
20. Martin RL, Monticelli F, Brackett WW, Loushine RJ, Rockman RA, Ferrari M, Pashley DH, Tay FR (2007)

Sealing properties of mineral trioxide aggregate orthograde apical plugs and root fillings in an in vitro apexification model. *J Endod* **33**, 272–5.
21. Boutsioukis C, Noula G, Lambrianidis T (2008) Ex vivo study of the efficiency of two techniques for the removal of mineral trioxide aggregate used as a root canal filling material. *J Endod* **34**, 1239–42.
22. Budig CG, Eleazer PD (2008) In vitro comparison of the setting of dry ProRoot MTA by moisture absorbed through the root. *J Endod* **34**, 712–4.
23. American Society for Testing Materials (2000) Standard test method for time and setting of hydraulic cement by Vicat needle, vol 04.01, Easton, USA, pp 179–81.
24. American Society for Testing Materials (2000) Standard test method for normal consistency of hydraulic cement, vol 04.01, Easton, USA, pp 175–6.
25. American Society for Testing Materials (1993) Standard test method for compressive properties of rigid plastics, vol 08.01, Easton, USA, pp 204–10.
26. Greenspan DC, Zhong JP, LaTorre GP (1994) Effect of surface area to volume ratio in vitro surface reactions of bioactive glass particulates. In: Andresson ŎH, Yli-Urpo A (eds) *Bioceramics* vol. 7, Turku, Finland, pp 55–60.
27. Zhao W, Wang J, Zhia W, Wang Z, Chang J (2005) The self-setting properties and in vitro bioactivity of tricalcium silicate. *Biomaterials* **26**, 6113–21.
28. Saboori A, Rabiee M, Moztarzadeh F, Sheikhi M, Tahriri M, Karimi M (2008) Synthesis, characterization and in vitro bioactivity of sol-gel-derived SiO_2–CaO–P_2O_5–MgO bioglass. *Mater Sci Eng C* **29**, 335–40.
29. Sun J, Li Y, Li L, Zhao W, Li L, Gao J, M, Ruan Shi J (2008) Functionalization and bioactivity in vitro of mesoporous bioactive glasses. *J Non Cryst Solids* **354**, 3799–805.
30. Salman SM, Salama SN, Darwish H, Abo-Mosallam HA (2008) In vitro bioactivity of glass–ceramics of the $CaMgSi_2O_6$–$CaSiO_3$–$Ca_5(PO_4)_3F$–Na_2SiO_3 system with TiO_2 or ZnO additives. *Ceram Int* **35**, 1083–93.
31. Ooms EM, Wolke JGC, van de Heuvel MT, Jeschke B, Jansen JA (2003) Histological evaluation of the bone response to calcium phosphate cement implanted in cortical bone. *Biomaterials* **24**, 989–1000.
32. Atsuro Y, Satoru Y, Takao KA, Takao KO, Masanori N (2002) Development of calcium phosphate cement using chitosan and citric acid for bone substitute materials. *Biomaterials* **23**, 1091–101.
33. Flautre B, Delecourt C, Blary MC, Van Landuyt P, Lemaître J, Hardouin P (1999) Volume effect on biological properties of a calcium phosphate hydraulic cement: experimental study in sheep. *Bone* **25**, 35S–9S.
34. Carey LE, Xu HHK, Simon CG, Takagi S, Chow LC (2005) Premixed rapid-setting calcium phosphate composites for bone repair. *Biomaterials* **26**, 5002–14.
35. Oreffo ROC, Driessens FCM, Planell JA, Triffitt JT (1998) Growth and differentiation of human bone marrow osteoprogenitors on novel calcium phosphate cements. *Biomaterials* **19**, 1845–54.
36. Gómez E, Martín M, Arias J, Carceller F (2005) Clinical applications of Norian SRS (calcium phosphate cement) in craniofacial reconstruction in children: Our experience at Hospital La Paz since 2001. *J Oral Maxillofac Surg* **63**, 8–14.
37. Gbureck U, Grolms O, Barraletb JE, Groverb LM, Thulla R (2003) Mechanical activation and cement formation of b-tricalcium phosphate. *Biomaterials* **24**, 4123–31.
38. Ratier A, Gibson IR, Best SM, Freche M, Lacout JL, Rodriguez F (2001) Setting characteristics and mechanical behaviour of a calcium phosphate bone cement containing tetracycline. *Biomaterials* **22**, 897–901.
39. Mariam Z, Anita L, Pierrette B, Jean G (2001) A self-setting single-component calcium phosphate cement. *Biomaterials* **22**, 1933–7.
40. Lea FM (1976) *The Chemistry of Cement and Concrete*, 3rd edn, Edward Arnold, London, pp 177–210.
41. Peitl O, Zanotto ED, Hench LL (2001) Highly bioactive P_2O_5-Na_2O-CaO-SiO_2 glass-ceramics. *J Non Cryst Solids* **292**, 115–26.
42. Jones JR, Ehrenfried LM, Hench LL (2006) Optimising bioactive glass scaffolds for bone tissue engineering. *Biomaterials* **27**, 964–73.
43. Nilsson M, Fernández E, Sarda S, Lidgren L, Planell JA (2002) Characterization of a novel calcium phosphate/sulfate bone cement. *J Biomed Mater Res* **61**, 600–7.
44. Cabañas MV, Rodríguez-Lorenzo LM, Vallet-Regí M (2002) Setting behavior and in vitro bioactivity of hydroxyapatite/calcium sulfate cements. *Chem Mater* **14**, 3550–5.
45. Gallego D, Higuita N, Garcia F, Ferrell N, Hansford DJ (2008) Bioactive coating on Portland cement substrate: Surface precipitation of apatite-like crystal. *Mater Sci Eng C* **28**, 347–52.
46. Leonardo RT, Consolaro A, Carlos IZ, Leonardo MR (2000) Evaluation of cell culture cytotoxicity of five root canal sealers. *J Endod* **26**, 328–30.
47. Al-Awadhi S, Spears R, Gutmann JL, Opperman LA (2004) Cultured primary osteoblast viability and apoptosis in the presence of root canal sealers. *J Endod* **30**, 527–33.