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Effect of tricalcium aluminate on the properties of tricalcium silicate–tricalcium aluminate mixtures: setting time, mechanical strength and biocompatibility

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Abstract

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Aim To prepare biphasic mixtures by adding $Ca_3Al_2O_6$ into Ca_3SiO_5 and to evaluate the effect of $Ca_3Al_2O_6$ on physical and *ex vivo* biological properties of the Ca_3SiO_5/$Ca_3Al_2O_6$ mixtures derived from mineral trioxide aggregate (MTA).

Methodology Combinations of Ca_3SiO_5 and $Ca_3Al_2O_6$ (0, 5%, 10% and 15%) powders were mixed with deionized water. After hydration, setting time, compressive strength, *ex vivo* bioactivity and biocompatibility of each mixture were investigated and compared to pure Ca_3SiO_5.

Results With the addition of $Ca_3Al_2O_6$ from 0% to 15%, the initial setting time and final setting time of the Ca_3SiO_5/$Ca_3Al_2O_6$ mixtures decreased from 110 to 43 min and from 220 to 97 min, respectively ($P \leq 0.05$). However, the compressive strength increased from 6.75 to 16.20 MPa after one day ($P \leq 0.05$) and from 17.73 to 29.13 Mpa after 28 days. Furthermore, the mixtures with 10% $Ca_3Al_2O_6$ or less had similar bioactivity and biocompatibility when compared to the pure Ca_3SiO_5.

Conclusions The addition of $Ca_3Al_2O_6$ into Ca_3SiO_5 accelerated the hydration process, reduced the setting time and improved the compressive strength. Furthermore, these mixtures were bioactive and biocompatible and had a stimulatory effect on the L929 cell growth when the content of $Ca_3Al_2O_6$ was below 10%. Therefore, the mixtures with 10% $Ca_3Al_2O_6$ produced the best compromise between hydration and *ex vivo* biological properties.

Keywords: bioactivity, biocompatibility, mineral trioxide aggregate, tricalcium silicate, tricalcium aluminate.

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Introduction

Mineral trioxide aggregate (MTA), initially developed as a root-end dental filling material (Lee *et al.* 1993) and then widely used in endodontics (Schwartz *et al.* 1999, Torabinejad & Chivian 1999, Roberts *et al.* 2008), is composed of a refined Portland cement which consists of tricalcium silicate (Ca_3SiO_5, C_3S), dicalcium silicate (Ca_2SiO_4), tricalcium aluminates ($Ca_3Al_2O_6$, C_3A) and tetracalcium aluminoferrite, bismuth oxide for radiopacity and other trace amounts of mineral oxides such as SiO_2, CaO, MgO, K_2SO_4 and Na_2SO_4 (Roberts *et al.* 2008). Previous studies have demonstrated that MTA has superior sealing ability and biocompatibility to traditional dental materials (Lee *et al.* 1993, Koh *et al.* 1998, Osorio *et al.* 1998, Cintra *et al.* 2006). However, the long setting time (3–4 h) is one of the major drawbacks of this material (Roberts *et al.* 2008). As a

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result, the search for a new cement with reduced setting time whilst having similar biocompatibility and mechanical properties as MTA is underway. For this purpose, studies to identify the function (such as the contribution to hydration process, the mechanical strength and the biocompatibility) of each component in MTA are necessary.

Ca_3SiO_5 is the main constituent of MTA and also the main raw material in Portland cement. Ca_3SiO_5 alone, which has excellent *in vitro* bioactivity and biocompatibility (Zhao *et al.* 2005), can react with an aqueous phase at room/body temperature to form injectable pastes. Calcium silicate hydrates (C-S-H) are the main hydrated phases, which possess a nano-crystalline structure and contribute to the self-setting properties and the spontaneous development of strength on hydration (Jiang *et al.* 1995, 1996, Zhao *et al.* 2005). Although Ca_3SiO_5 is known for its ability to be mainly responsible for the initial set and strength development of Portland cement (Taylor 1997), single-phase Ca_3SiO_5 has several undesirable shortcomings such as long setting time (3–4 h) which is similar to MTA and low mechanical strength at the early stage (Zhao *et al.* 2005). $Ca_3Al_2O_6$ is well known to have the fastest hydration rate amongst the main components of Portland cement so that it may accelerate the hydration process and improve the short-term compressive strength of $Ca_3SiO_5/Ca_3Al_2O_6$ composites when compared with that of pure Ca_3SiO_5 (Taylor 1997, Black *et al.* 2006). In this study, two components of MTA, Ca_3SiO_5 and $Ca_3Al_2O_6$, were selected to form biphasic mixtures, and the effect of $Ca_3Al_2O_6$ on the setting time and mechanical strength of the $Ca_3SiO_5/Ca_3Al_2O_6$ mixtures were investigated. In addition, the *ex vivo* bioactivity and biocompatibility of such mixtures were evaluated to determine whether $Ca_3Al_2O_6$ could also affect these properties.

Materials and methods

Preparation and characterization

The composite powders consisted of two components, Ca_3SiO_5 and $Ca_3Al_2O_6$. Ca_3SiO_5 was prepared by the sol-gel method as described previously (Zhao & Chang 2004). Briefly, 0.5 mol Si $(OC_2H5)_4$ (tetraethyl orthosilicate) and nitric acid as catalyst were added in 200 mL water under continuous stirring. A volume of 1.5 mol Ca $(NO_3)_2{\cdot}4H_2O$, as the calcium precursor, was added to the solution. Then, the solution was maintained at 60 °C until gelation occurred. The gel was dried at 120 °C and then calcined at 1450 °C for 8 h. The resultant powders were ground and sieved through 300-mesh (<52 μm) for further experiments. $Ca_3Al_2O_6$ was prepared using a solution-polymerization route based on poly(vinyl alcohol) (PVA AH-26, 50–70 $mm^2\ s^{-1}$; Sinopham Chemical Reagent Co. Ltd, Shanghai, China) as the polymer carrier (Lee *et al.* 1999). Briefly, 0.2 mol Al $(NO_3)_3{\cdot}9H_2O$ and 0.3 mol Ca $(NO_3)_2{\cdot}4H_2O$ were dissolved in water and then mixed with 5 wt% PVA aqueous solution (0.3 mol PVA). Water was evaporated during heating and the resultant gel-type precursors were further dried at 120 °C overnight. Then, the completely dried organic/inorganic precursors were calcined at 1350 °C for 3 h and the powders obtained were also sieved through 300-mesh (<52 μm).

To prepare $Ca_3SiO_5/Ca_3Al_2O_6$ mixtures, $Ca_3Al_2O_6$ powders (0–15 wt %) were uniformly mixed with Ca_3SiO_5 and then the resultant biphasic powders were manually mixed with deionized water at the liquid to powder (L/P) ratio of 0.8 mL g^{-1}. The mixtures were stirred to form homogeneous pastes within 60 s, transferred into teflon moulds to form standard test cylinders with 6 mm in diameter and 12 mm in height without compression and finally cured in a 100% humidity environment at 37 °C. After 1, 7, 14, 21 and 28 days, the cylinders were soaked in acetone (100%) for 2 h to arrest hydration and air-dried (Zhao *et al.* 2005). The compressive strength was measured at a loading rate of 0.5 mm min^{-1} using a universal testing machine (Instron-1195; Instron, Norwood, MA, USA) according to ASTM D695-91. Six replicates were carried out for each group and the results were expressed as mean ± standard deviation (mean ± SD).

The phase composition of as-prepared cylinders after 7 days was characterized by X-ray diffraction (XRD; Geigerflex, Rigaku Co., Japan) with Cu (Ka) radiation, operating at 40 kV and 100 mA. The cross-section of the same samples was observed by scanning electron microscopy (SEM, JSM-6700F; JEOL, Tokyo, Japan).

Setting time

Initial and final setting times were measured with the Vicat needle according to ISO9597-1989E. The initial setting time was defined as the time necessary so that the light needle (280 g, Ø1.13 mm) plunges into the paste and has a span of 5 ± 1 mm to the tube bottom. The final setting time was defined as the time necessary so that the heavy needle (350 g, Ø2.0 mm) no longer leaves a perceptible circular indentation on the surface

of the paste. Five replicates were carried out for each measurement and the results were expressed as mean ± standard deviation (mean ± SD).

Soaking in SBF

The simulated body fluid (SBF) was prepared according to the procedure described previously (Kokubo 1990). The 7-day-set discs (6 mm in diameter and 2 mm in thickness) were soaked in SBF solution at 37 °C in an agitated water bath for 7 days with a surface-area-to-volume ratio of 0.1 cm^{-1} (Zhao *et al.* 2005). Then, the discs were gently rinsed with deionized water to remove the SBF solutions followed by drying at room temperature. The morphological variations on the surfaces of the discs were characterized by XRD and SEM after soaking in SBF.

MTT assay

The cell proliferation assay was performed by the extraction method with mouse fibroblast cell line L929 according to the method reported in ISO 10993-5. The 7-day-set samples were crushed to powders and sieved through 300-mesh (52 μm) for further experiments. The dissolution extracts were prepared by adding these powders to Roswell Park Memorial Institute 1640 (RPMI 1640; Gibco, Carlsbad, CA, USA) cell culture medium for 1 day at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air without agitation. Ratios between the powder weight (mg) and the medium volume (mL) were 3.125, 6.25, 10, 25, 50, 100 and 200 mg mL^{-1}. After incubation, the mixture was centrifuged and the supernatant collected.

The cell suspension was adjusted to a density of 1×10^4 cell mL^{-1}, and 100- μL cell suspension was added to each well of a 96-well plate and incubated for 24 h. The culture medium was then removed and replaced by 50 μL of extracts and 50 μL of RPMI 1640 medium supplemented with 20% FCS. The medium supplemented with 10% FCS without addition of extracts was used as a negative control ($Ctrl^-$). The medium supplemented with 0.2% Trion-X100 was used as a positive control ($Ctrl^+$). The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method was used to assess the cell proliferation levels (Cory *et al.* 1991). This assay relies upon the ability of living cells to reduce a tetrazolium salt into a soluble coloured formazan product. After incubating at 37 °C and 5% CO_2 for 6 days, 100 μL of 0.5 mg mL^{-1} MTT solution was added and incubated for 4 h at 37 °C. Then, 100 μL dimethyl sulfoxide (DMSO) was added to each well, the plate was shaken for 5 min and the optical density (OD) at 490 nm was measured with an enzyme-linked immunoadsorbent assay (ELISA) plate reader (ELX800; Bio-Tek, Winooski, VT, USA).

Statistical methods

The data were analysed using standard analysis of Student's t test and expressed as means ± standard deviation (SD). A P value <0.05 was considered statistically significant.

Results

Characterization of cements

After setting for 7 days at 37 °C and 100% relative humidity, the $Ca_3SiO_5/Ca_3Al_2O_6$ specimen cylinders were characterized by XRD (Fig. 1). Calcium hydroxide [$Ca(OH)_2$] and calcium silicate hydrate (C-S-H, $2\theta = 29.04°$) were identified in all patterns because of the hydration of Ca_3SiO_5 (Zhao *et al.* 2005). Some peaks for non-reacted Ca_3SiO_5 were also observed. Meanwhile, $Ca_3Al_2O_6$ also reacted with water. When the content of $Ca_3Al_2O_6$ in the $Ca_3SiO_5/Ca_3Al_2O_6$ mixtures was more than 10%, the peaks for $Ca_3Al_2(OH)_{12}$ (C_3AH_6, JCPDS 24-0217) and $Ca_4Al_2O_7 \cdot 19H_2O$ (C_3AH_{19}, JCPDS 14-0628) were obvious, which were the two main hydration products of $Ca_3Al_2O_6$ (Black *et al.* 2006).

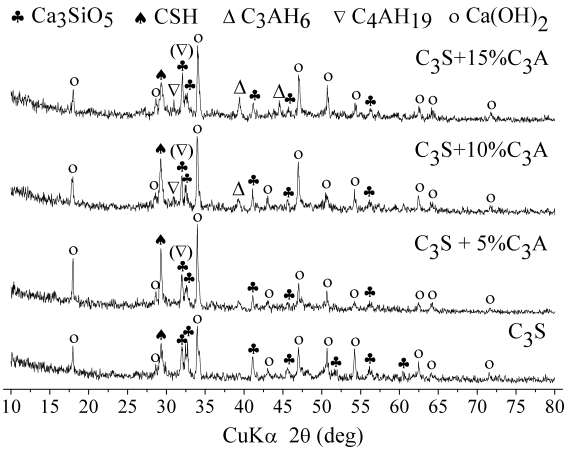

Figure 1 XRD patterns of the $Ca_3SiO_5/Ca_3Al_2O_6$ specimens after setting for 7 days.

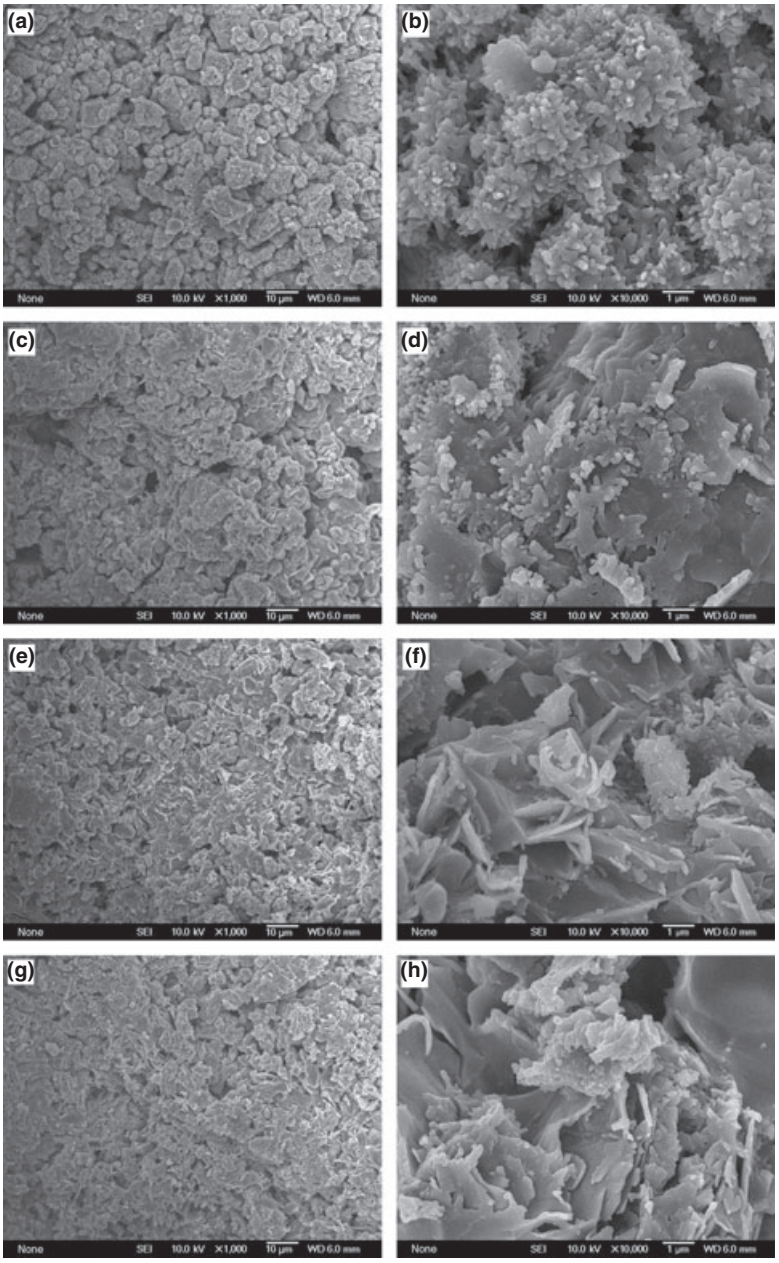

Figure 2 SEM micrographs of the cross-section of the $Ca_3SiO_5/Ca_3Al_2O_6$ specimens after setting for 7 days: (a, b) Ca_3SiO_5; (c, d), Ca_3SiO_5 + 5%$Ca_3Al_2O_6$; (e, f), Ca_3SiO_5 + 10%$Ca_3Al_2O_6$; (g, h), Ca_3SiO_5 + 15%$Ca_3Al_2O_6$.

Apart from XRD analysis, SEM was used to observe the cross-section of cylinders after setting for 7 days (Fig. 2). Similar fracture morphologies with an interconnected microporous structure were observed. However, when the weight ratio of $Ca_3Al_2O_6$ increased up to 10% or even more, it was obvious that the Ca_3SiO_5/$Ca_3Al_2O_6$ mixtures possessed smaller pores and consequently compact structure. Furthermore, it was observed from the corresponding pictures at higher magnification that a large number of thick lamellar crystallites appeared, with clustered crystals becoming less notable when contents of $Ca_3Al_2O_6$ were higher.

Compressive strength and setting time

Compared to pure Ca_3SiO_5, the addition of $Ca_3Al_2O_6$ increased mechanical strength of the Ca_3SiO_5/$Ca_3Al_2O_6$ mixtures (Fig. 3a). With more $Ca_3Al_2O_6$, the strength increased at a higher rate; though, the accelerating effect on the increase in strength was less

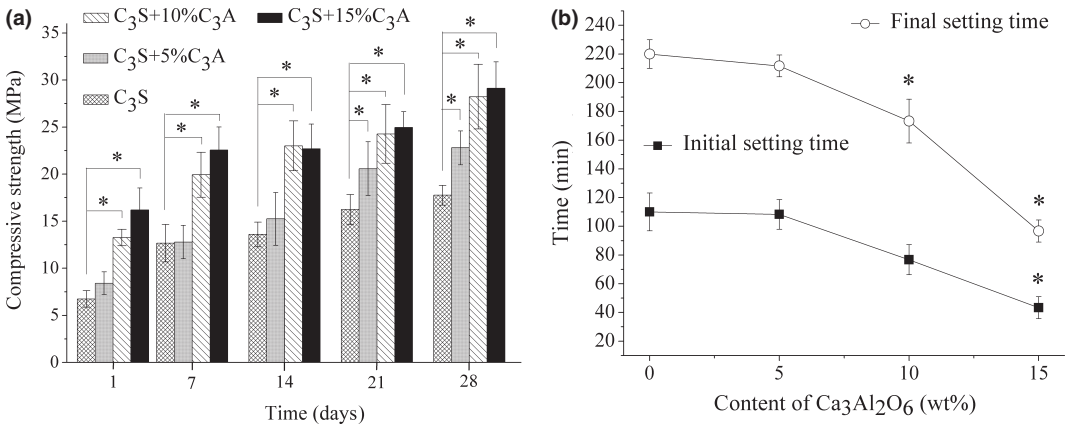

Figure 3 The effect of $Ca_3Al_2O_6$ on (a) compressive strength and (b) setting time of the $Ca_3SiO_5/Ca_3Al_2O_6$ specimens. *Denotes statistically significant difference from the specimen without $Ca_3Al_2O_6$ ($P < 0.05$, Student's t test).

obvious after 14 days when the $Ca_3Al_2O_6$ percentage was above 10%. The 21-day and 28-day strength values of the specimen with 5% $Ca_3Al_2O_6$ were significantly higher than those of pure Ca_3SiO_5 ($P < 0.05$); though, the 14-day and earlier strength values of the specimen did not reveal significant differences when compared with those of Ca_3SiO_5. However, the specimens with 10% or 15% $Ca_3Al_2O_6$ had a remarkable effect on promoting strength development from an early stage. For example, the compressive strength increased from 6.75 to 16.20 MPa after one day and from 17.73 to 29.13 Mpa after 28 days when the content of $Ca_3Al_2O_6$ was 15%.

$Ca_3Al_2O_6$ also had an accelerating effect on the hydration rate of the mixtures when compared with that of pure Ca_3SiO_5 (Fig. 3b). With the addition of $Ca_3Al_2O_6$ from 0% to 15%, the initial and final setting time of the $Ca_3SiO_5/Ca_3Al_2O_6$ mixtures decreased significantly from 110 to 43 min and from 220 to 97 min, respectively ($P < 0.05$). However, this accelerating effect on the hydration process was not remarkable until the $Ca_3Al_2O_6$ percentage reached 10% and above.

Evaluation of cement discs soaking in SBF

After soaking in SBF for 7 days, phase compositions on disc surfaces of the $Ca_3SiO_5/Ca_3Al_2O_6$ composites were analysed by XRD (Fig. 4). The characteristic peaks for hydroxyapatite (HA, JCPDS 25-0166) appeared in all patterns, which suggested that HA was precipitated on the surfaces of all specimens. In addition, C-S-H and calcium carbonate ($CaCO_3$) were identified, which

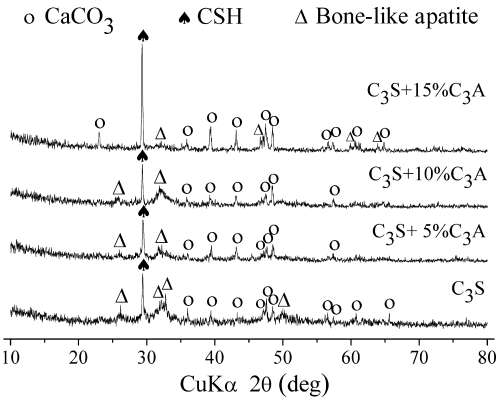

Figure 4 XRD patterns of the surfaces of the $Ca_3SiO_5/Ca_3Al_2O_6$ specimens after soaking in simulated body fluid for 7 days.

formed during the hydration of Ca_3SiO_5 and the transformation of hydration-derived Ca $(OH)_2$ in SBF solutions, respectively (Zhao *et al.* 2005). When compared with the pure Ca_3SiO_5, the $Ca_3SiO_5/Ca_3Al_2O_6$ mixtures with 5 or 10% $Ca_3Al_2O_6$ presented very similar XRD patterns. However, when $Ca_3Al_2O_6$ percentage was up to 15%, the peak intensity of HA for this specimen was lower than that for the other three. From the SEM images (Fig. 5g,h), the HA crystallites grew slowly and consequently, the HA layer did not cover the surface. In contrast, numerous HA crystallites deposited on surfaces of the pure Ca_3SiO_5 (Fig. 5a) and the specimen with 5% $Ca_3Al_2O_6$ (Fig. 5c). Higher magnification SEM micrographs (Fig. 5b,d) revealed that the particles of HA were lathlike and many of them

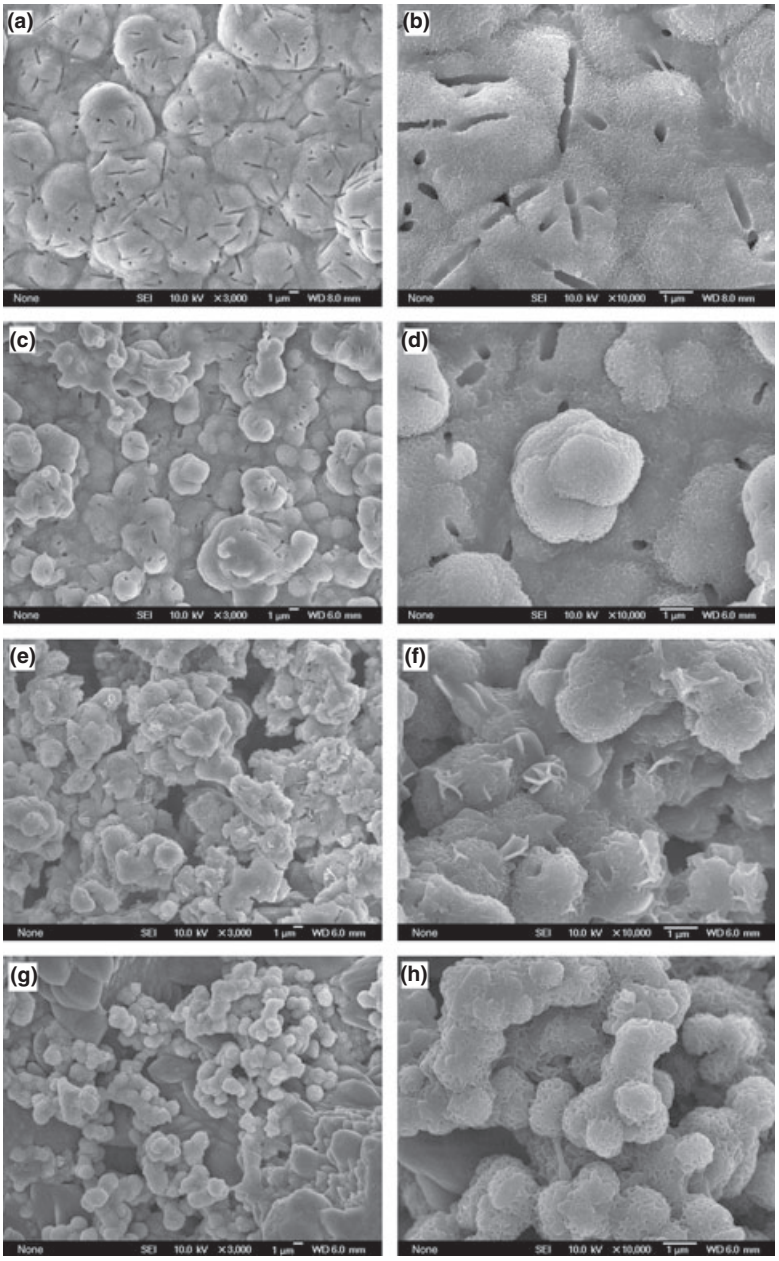

Figure 5 SEM micrographs of the surfaces of the $Ca_3SiO_5/Ca_3Al_2O_6$ specimens after soaking in simulated body fluid for 7 days: (a, b) Ca_3SiO_5; (c, d), Ca_3SiO_5 + 5%$Ca_3Al_2O_6$; (e, f), Ca_3SiO_5 + 10%$Ca_3Al_2O_6$; (g, h), Ca_3SiO_5 + 15%$Ca_3Al_2O_6$.

formed agglomerates with typical bone-like apatite morphology. Even if the $Ca_3Al_2O_6$ percentage was increased to 10%, a layer of HA covered the surface of such specimen (Fig. 5e) and the typical apatite morphology was still clearly observed (Fig. 5f).

In vitro biocompatibility

The MTT assay demonstrated that the dissolution extracts of the $Ca_3SiO_5/Ca_3Al_2O_6$ mixtures with different amounts of $Ca_3Al_2O_6$ (0–15%) did not show significant cytotoxicity against L929 cells after incubation for 6 days within the concentration range between 3.125 to 100 mg mL^{-1} (Fig. 6). Furthermore, the OD values of pure Ca_3SiO_5 were significantly higher than those of the negative control ($P < 0.05$) over the whole range of test concentrations except 200 mg mL^{-1}, which suggested that the extracts of pure Ca_3SiO_5 had a stimulatory effect on cell growth when compared with the negative control. With a less content of

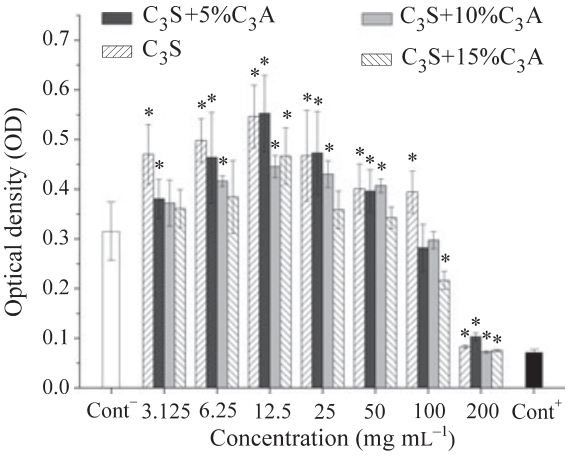

Figure 6 The cell proliferation after culturing in the dissolution extracts of the 7-day-set specimens (Ca_3SiO_5; Ca_3SiO_5 + 5%$Ca_3Al_2O_6$; Ca_3SiO_5 + 10%$Ca_3Al_2O_6$; Ca_3SiO_5 + 15%$Ca_3Al_2O_6$) for 6 days. *Denotes significant difference from the ctrl$^-$, $P < 0.05$.

$Ca_3Al_2O_6$ (5%), the extracts of this specimen had a similar stimulatory effect on cell growth to that of pure Ca_3SiO_5. When the content of $Ca_3Al_2O_6$ increased to 10%, the specimens were non-cytotoxic almost in the whole test range, and its OD values were significantly higher than those of the negative control as the concentration varied from 6.25 to 50 mg mL^{-1}, which meant that the extracts of such composite could also activate L929 cell proliferation in certain concentration ranges. Even if the content of $Ca_3Al_2O_6$ increased up to 15%, the specimen did not reveal an inhibitory effect on cell growth at lower concentrations below 100 mg mL^{-1}.

Discussion

The goal of the present study was to identify functions of Ca_3SiO_5 and $Ca_3Al_2O_6$, two important components of MTA, and to investigate the effect of $Ca_3Al_2O_6$ on properties of $Ca_3SiO_5/Ca_3Al_2O_6$ mixtures. Ca_3SiO_5 as the main constituent of Portland cement has good *in vitro* bioactivity and hydraulic properties, which are not affected by moisture. However, the single-phase Ca_3SiO_5 as bone cement has a long setting time (3–4 h) which is similar to that of MTA and poor mechanical strength at the early stage of setting (Zhao *et al.* 2005). $Ca_3Al_2O_6$ is well known as the most reactive part of Portland cement and thus, it is reasonable to assume that the addition of $Ca_3Al_2O_6$ into Ca_3SiO_5 may result in a mixture with decreased setting time and enhanced early stage compressive strength when compared with pure Ca_3SiO_5 (Taylor 1997). Although higher magnification SEM micrographs (Fig. 2b,d,f,h) revealed typical clustered and lamellar morphologies for the hydration products of Ca_3SiO_5 and $Ca_3Al_2O_6$ (Breval 1976, 1977), respectively, fracture morphologies of all specimen formulations were similar from the low magnification images (Fig. 2a,c,e,g), suggesting that the addition of $Ca_3Al_2O_6$ did not alter the original integrity of Ca_3SiO_5 cements. As is well known in cement chemistry, it is necessary for hydraulic cements to have a microporous network to enhance hydration and this depends on the porosity distribution. The mechanical strength of the cement is closely related to the porosity distribution which can be assessed using the size distribution of sectioned pore areas and characterized by SEM, Decreasing the porosity distribution of hydraulic cements results in increasing compressive strength (Taylor 1997, Dunne *et al.* 2003, Fleming *et al.* 2003). As observed from the SEM images (Fig. 2), the $Ca_3SiO_5/Ca_3Al_2O_6$ mixtures, especially with 10% or more $Ca_3Al_2O_6$ (Fig. 2e,f), had a more compact structure because of the decreased porosity distribution when compared with pure Ca_3SiO_5 (Fig. 2a), which suggests that the $Ca_3SiO_5/Ca_3Al_2O_6$ mixtures hydrated more rapidly and consequently had a shorter setting time and higher mechanical strength. As shown in Fig. 3, an increase in the weight ratio of $Ca_3Al_2O_6$ in the $Ca_3SiO_5/Ca_3Al_2O_6$ mixtures resulted in a decrease in setting time and an increase in compressive strength, especially at the initial stage of setting. Previous studies have shown that ProRoot MTA-containing calcium sulphate had a much longer setting time (3–4 h) than MTA Angelus (about 15 min) without calcium sulphate (Roberts *et al.* 2008, Santos *et al.* 2008), The calcium sulphate acts as a setting retarder because it preferentially reacts with $Ca_3Al_2O_6$ to produce a layer on the cement surface which delays the hydration process of MTA (Dammaschke *et al.* 2005). Meanwhile, the present study revealed that the $Ca_3Al_2O_6$ had a critical role in the early hydration properties of the $Ca_3SiO_5/Ca_3Al_2O_6$ mixtures and had an accelerating effect on the hydration process of such mixtures in the absence of calcium sulphate. Therefore, $Ca_3Al_2O_6$ was demonstrated to be a reactive component for the hydration process. In addition, it is known that the condensation pressure during the placement of the cement in endodontic treatment, such as root-end filling and surgical repair of perforations, may affect the properties and performance

of dental materials such as amalgam and MTA (Lussi *et al.* 1995, Nekoofar *et al.* 2007). However, the primary goal of the present study was to investigate the effect of the amount of the $Ca_3Al_2O_6$ on the setting time and strength of the $Ca_3SiO_5/Ca_3Al_2O_6$ mixtures under fixed setting conditions. Therefore, the effect of the condensation pressure was not considered. Nevertheless, further studies on the effect of condensation pressure on properties of the $Ca_3SiO_5/Ca_3Al_2O_6$ mixtures need to be considered in development of clinical applicable cements.

HA possesses excellent biocompatibility, and the HA surface layer plays an essential role in the formation, growth and maintenance of the tissue–biomaterial interface (Kokubo 1990, LeGeros 1991). Sarkar *et al.* (2005) demonstrated that the HA layer deposited on the surface of MTA after exposure to physiological solution facilitated its integration with dentine. In previous studies, pure Ca_3SiO_5 revealed excellent *in vitro* bioactivity by induction of HA formation on its surface when immersed in SBF (Zhao *et al.* 2005). In the present study, the results of XRD (Fig. 4) and SEM (Fig. 5) analyses verified that the $Ca_3SiO_5/Ca_3Al_2O_6$ mixtures could induce the formation of a HA layer on its surface in SBF suggesting good *in vitro* bioactivity. Although the addition of $Ca_3Al_2O_6$ partially affected the apatite induction rate, the $Ca_3SiO_5/Ca_3Al_2O_6$ composites with 10% $Ca_3Al_2O_6$ or less were still bioactive in SBF. Zinc oxide eugenol (ZOE) sealers have been used for many decades as traditional root canal filing materials. Clinically, their biocompatibility is generally considered good. However, ZOE sealers may trigger moderate to severe inflammation in rats after subcutaneous implantation (Kolokouris *et al.* 1998). Meanwhile, Ca–Si biomaterials possess excellent biocompatibility indicated by the induction of the formation of bone-like apatite, which could stimulate deposition of mineralized tissue on their surface and further integrate with surrounding tissues *in vivo* (Hench & Paschall 1973, Xue *et al.* 2005). Therefore, the $Ca_3SiO_5/Ca_3Al_2O_6$ mixtures with good apatite-forming ability in physiological solution may have similar behaviours *in vivo*, which benefit the formation of the dentine–material interface (Sarkar *et al.* 2005). However, this assumption needs to be confirmed by further *in vivo* studies.

According to a previous study, the dissolution extracts of 7-day-set Ca_3SiO_5 could stimulate L929 cell proliferation (Zhao *et al.* 2005). Other studies also demonstrated that the dissolution extracts of bioactive silicate materials such as bioactive glasses (Xynos *et al.* 2001, Valério *et al.* 2004) and calcium silicate bone cements (Ding *et al.* 2009, Huan & Chang 2009) could stimulate cell proliferation. This phenomenon was probably because of the dissolution of silicate ions which could stimulate collagen type I synthesis and osteoblastic differentiation in human osteoblast-like cells (Reffitt *et al.* 2003, Sun *et al.* 2006). MTA is a silicate cement-based biomaterial, and studies have reported that MTA was non-cytotoxic *ex vivo* and could support cell adhesion and proliferation (Koh *et al.* 1997, Mitchell *et al.* 1999, Zhu *et al.* 2000, Pelliccioni *et al.* 2004, Perinpanayagam & Al-Rabeah 2009) and even to stimulate gene expression in osteoblast (Mitchell *et al.* 1999, Perinpanayagam & Al-Rabeah 2009). Furthermore, the *in vivo* studies demonstrated that MTA was able to stimulate osteogenesis in test animals (Torabinejad *et al.* 1998, Torabinejad & Chivian 1999), with little inflammation (Saidon *et al.* 2003) or any adverse effect on microcirculation of the connective tissue (Masuda *et al.* 2005). Therefore, silicate-based MTA was biocompatible and even bioactive both *in vitro* and *in vivo*. In the current study, the $Ca_3SiO_5/Ca_3Al_2O_6$ mixtures with 10% $Ca_3Al_2O_6$ or less were non-cytotoxic and presented a stimulatory effect on the L929 cell growth in certain concentration ranges, whilst the specimens with 15% $Ca_3Al_2O_6$ were also non-cytotoxic in a wide test concentration range. However, the positive effect of its extracts on cell growth was limited in a narrow concentration range between 12.5 and 25 mg mL^{-1}, so 10% $Ca_3Al_2O_6$ or less appeared to be a good choice from the viewpoint of *in vitro* biocompatibility. Similarly, the $Ca_3Al_2O_6$ weight percentage in ProRoot MTA without bismuth oxide is 10.62% (Dammaschke *et al.* 2005), which suggests that the 10% $Ca_3Al_2O_6$ in the $Ca_3SiO_5/Ca_3Al_2O_6$ mixtures is within safe range.

Conclusions

$Ca_3Al_2O_6$ played a critical role in the early hydration properties of the $Ca_3SiO_5/Ca_3Al_2O_6$ mixtures. The addition of $Ca_3Al_2O_6$ to Ca_3SiO_5 could reduce the setting time and improve the compressive strength of such mixtures which also demonstrated good apatite-forming ability in SBF if the amount of $Ca_3Al_2O_6$ was <10%. Moreover, dissolution extracts of the specimen mixed with 10% $Ca_3Al_2O_6$ or less had a stimulatory effect on L929 cell growth in certain concentration ranges. Therefore, the $Ca_3SiO_5/Ca_3Al_2O_6$ mixtures with 10% $Ca_3Al_2O_6$ seemed to be a best compromise between setting time, compressive strength, *in vitro*

bioactivity and biocompatibility. However, further studies on sealing ability, radiopacity, antimicrobial activity and *in vivo* biocompatibility are required.

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