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Preparation and characterization of a degradable magnesium phosphate bone cement

Ying Yu[1], Chao Xu[1] and Honglian Dai[1,2]*

[1]State Key Laboratory of Advanced Technology for Materials Synthesis and Processing, Wuhan University of Technology, Wuhan 430070, PR China

[2]Biomedical Materials and Engineering Research Center of Hubei Province, Wuhan 430070, PR China

*Correspondence address. State Key Laboratory of Advanced Technology for Materials Synthesis and Processing, Wuhan University of Technology, Wuhan 430070, PR China. Tel: 18971519802; E-mail: daihonglian@whut.edu.cn

Abstract

A kind of degradable magnesium phosphate bone cement (MPBC) was fabricated by using the mixed powders of magnesium oxide (MgO), potassium dihydrogen phosphate (KH_2PO_4) and calcium dihydrogen phosphate ($Ca(H_2PO_4)_2.H_2O$). As $MgKPO_4$, the main product of MgO and KH_2PO_4 was alkaline, the $Ca(H_2PO_4)_2.H_2O$ was added to neutralize the alkali of the system. And the effects of $Ca(H_2PO_4)_2.H_2O$ on the performance of MPBC were discussed. The results showed that the adding of $Ca(H_2PO_4)_2.H_2O$ extended the setting time, which was about 6 min to 18 min. The compressive strength increased first and then decreased, and maximum value reached 31.2 MPa after setting for 24 h without any additional pressure. The MPBC was degradable in Tris–HCl solution, and the extracts of the cytotoxicity assay showed that the MPBC had good biocompatibility, indicating that the MPBC had good biodegradable and biocompatible properties.

Keywords: magnesium phosphate bone cement; degradable; biocompatibility

Introduction

Magnesium phosphate bone cement (MPBC) has attracted much attention in bone regeneration for its high initial strength, fast setting time and moderate degradation rate compared with calcium phosphate bone cement (CMPC) [1–3]. Traditional MPBC is mixed with dead burnt magnesium oxide (MgO) and ammonium dihydrogen phosphate ($NH_4H_2PO_4$) or diammonium phosphate (($NH_4)_2HPO_4$) as solid component, and the main reaction product is magnesium ammonium phosphate hexahydrate ($MgNH_4PO_4 \cdot 6H_2O$), known as struvite, a naturally existing crystal [4]. While the problem is that after or during setting they may release ammonia, which may cause cytotoxicity in the physiological environment [5]. However, it has been reported that Mg^{2+} irons released *in vivo* could increase osteoblast activity, meaning Mg^{2+} irons play an important role in bone regeneration [6–7]. Therefore, a study by Mestres and Ginebra is focused on the replacements of $NH_4H_2PO_4$ and ($NH_4)_2HPO_4$ [8]. On the other hand, potassium dihydrogen phosphate

(KH_2PO_4) is used to replace $NH_4H_2PO_4$ in civil engineering, which is called as magnesium potassium phosphate cement (MPP) [9–14]. When compared to $NH_4H_2PO_4$, KH_2PO_4 not only has smaller dissociation constant and lower solubility, resulted in controlling the reaction rate easier, but also does not produce unpleasant odour when reacts with water [15–16], and the final product is magnesium potassium phosphate hexahydrate ($MgKPO_4 \cdot 6H_2O$), which is isostructural with struvite and also a naturally existing mineral known as struvite-(K) [17–18]. Many researchers studied the engineering properties and reaction mechanisms of MPP [9,19–23]. However, few researches were about applications in MPP for bone repair. In this work, the alkali of $MgKPO_4 \cdot 6H_2O$ is discussed first, which had an adverse effect on the biocompatibility. And then $Ca(H_2PO_4)_2$ was added into the mixture of MgO and KH_2PO_4 to neutralize alkaline of $MgKPO_4 \cdot 6H_2O$. And the influences of $Ca(H_2PO_4)_2$ on setting time, compressive strength, degradation rate and biocompatibility are also discussed.

Materials and Methods

Preparation of MPBC

MPBC composed of powders and cement liquid (deionized water), and the powders were composed of MgO, KH_2PO_4 and $Ca(H_2PO_4)_2 \cdot H_2O$. The MgO was heated to $1600\,^{\circ}C$ for $1.5\,h$, and the particles were grounded to be smaller than $2.68\,\mu m$. The KH_2PO_4 and $Ca(H_2PO_4)_2 \cdot H_2O$ were grounded and followed by sieving 140 mesh and 200 mesh, respectively. The biocement paste was formed by mixing MPBC powders with deionized water at a powder/liquid ratio of $1.8\,g/ml$, and placed into plexiglass moulds (size $\phi 10x10\,mm$) with no added pressure. After storing in water bath with the temperature at $37\,^{\circ}C$ and 100% relative humidity for $24\,h$, the hardened MPBC samples were obtained.

The MPBC sample hardened for $24\,h$ was characterized by X-ray diffraction (XRD; Rigaku Co., Japan) and the surface morphology/microstructure was examined by SEM (Zeiss Ultra Plus).

In order to test the pH of $MgKPO_4 \cdot 6H_2O$, four different mass ratio of MgO and KH_2PO_4, which were 1:3, 1:3.4, 1:3.8, 1:4, were chosen to synthesise $MgKPO_4 \cdot 6H_2O$. And when the ratio of MgO and KH_2PO_4 was 1:3, the $Ca(H_2PO_4)_2$ was added in and its effects on the performance of MPBC are discussed.

The pH determination of MPBC extracts

In order to determine the pH in MPBC extracts, hardened samples were soaked in physiological saline solution at a powder/liquid ratio of $0.2\,g/ml$ in tubes and shanked in a temperature oscillation box for $24\,h$. Then the supernatant was taken out and tested by pH meter.

Setting time and compressive strength

The setting time of MPBC was measured with a Vicat apparatus bearing a movable rod, weighing $300\,g$, and a $1\,mm$ needle. The setting time was the number of minutes elapsed from the start of mixing to the time that the needle failed to penetrate more than $1\,mm$ into MPBC paste. The average value was calculated with three tests.

After hardening for $24\,h$, the compressive strength was measured by a universal testing machine (MTS810, America) with a loading rate of $2\,mm/min$. Five samples were carried out for each group.

Degradation in Tris-HCl solution

The degradation of MPBC in Tris-HCl solution (pH $= 7.4$) was determined by the weight loss ratio at different time points. The hardened samples ($\phi 10x10\,mm$) were first dried at $50\,^{\circ}C$ [24] for $2\,h$, and the initial weight W_0, was recorded. Then, they were immersed in Tris-HCl solution in a temperature oscillation box at $37\,^{\circ}C$ and at a liquid/solid mass ratio of $20\,ml/g$. Then the solution was refreshed every 2 days. After soaking, the specimens were removed from the liquid, rinsed with distilled water and dried at $50\,^{\circ}C$ for $2\,h$. And the new weight W_t was also recorded. All the values presented were averages of three tests. The weight loss ratio was calculated as follows:

$$Weight\ loss\ ratios = (W_0 - W_t)/W_0 \times 100\%$$

Cell culture

Fibroblast cells (L929) were chosen. They were cultured in complete Roswell Park Memorial Institute 1640 (RPMI) containing 10% foetal bovine serum (FBS), 1% antibiotics (penicillin, streptomycin) and at $37\,^{\circ}C$ in a humidified incubator with 5% CO_2. The cells were harvested at confluence with 0.25% trypsin and seeded onto the disks, respectively, with an initial density of 2,000 cells per well in a 96-well plate and incubated at $37\,^{\circ}C/CO_2$. The medium was replaced every 3 days.

The cytotoxicity of MPBC

The extracts of MPBC were prepared according to the procedures reported in the literature [25]. Firstly, the solutions were obtained by adding sterilized powders into serum-free RPMI at a solid/liquid ratio of $0.2\,g/ml$. After incubation at $37\,^{\circ}C$ for $24\,h$, the mixture was centrifuged, and the supernatant was collected and then stored at $4\,^{\circ}C$ for further use.

The cytotoxicity of MPBC was evaluated by an MTT (3-(4,5-dimethylthiazol-2yl)-2,5-disphenyl-2H-tetrazolium-bromide) assay at day 1, day 3 and day 5. A $20\,\mu l$ MTT solution ($5\,mg\,ml^{-1}$) was added into each well and incubated for $4\,h$ at $37\,^{\circ}C/5\%\ CO_2$. Then, the medium was discarded and $200\,\mu l$ dimethylsulfoxide (DMSO) was added to dissolve purple crystals. The optical density (OD) of the solution was tested by a microplate reader on the first, third and fifth day at $490\,nm$.

Results and Discussion

The pH of $KMgPO_4$

Figure 1 shows the XRD and pH results when the MgO and KH_2PO_4 were at different mass ratios. The formula of X-ray diffraction intensity is shown in 3-1. It meant that the XRD diffraction intensity was not linear with the content of the phase. But when the samples were formed by substances possessing the same structure, it could result in the same parameters in 3-1. In this particular case, the intensity of the diffraction peak would increase with the increase of the phase content. All the samples were composed of MgO and $KMgPO_4$ (Fig. 1). The diffraction peak intensity of MgO decreased, while the diffraction peak intensity of $KMgPO_4$ increased with the increase of KH_2PO_4, meaning that MgO was totally transformed into $KMgPO_4$. And the pH got larger at the same time, proving that the $KMgPO_4$ was alkaline.

$$I = M \cdot Lp \cdot F^2 \cdot e^{-2M} \cdot A * (\theta) \cdot (PO) \qquad 3\text{-}1$$

M—Multiple factors, Lp—Lorentz polarization factor, F^2—Structural factor, e^{-2M}—Temperature factor, $A^*(\theta)$—Absorption factor, PO—Preferred orientation factor.
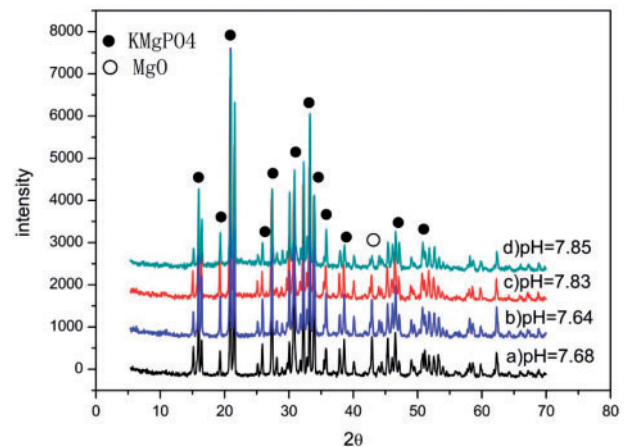

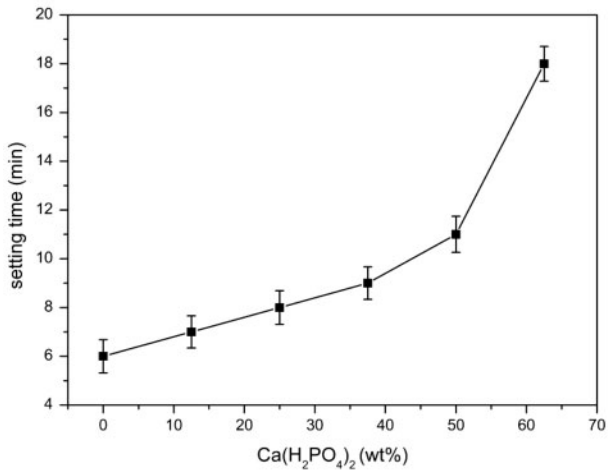**Figure 1.** the XRD and pH results. a) 1:3; b) 1:3.4; c) 1:3.8; d)1:4.

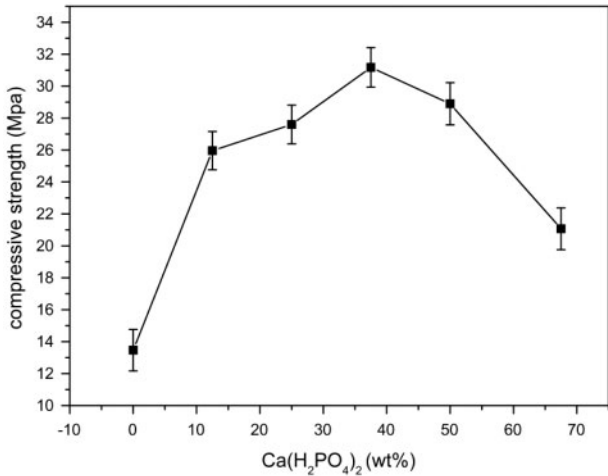**Figure 2.** Effect of $Ca(H_2PO_4)_2$ on the setting time.

Figure 3. Effect of $Ca(H_2PO_4)_2$ on the compressive strength.
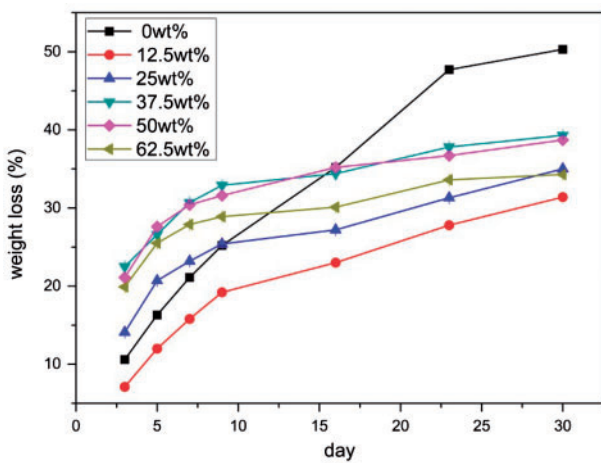
Figure 4. the SEM images of MPBC samples (1)0% $Ca(H_2PO_4)_2$ (2) 12.5% $Ca(H_2PO_4)_2$ (3) 25% $Ca(H_2PO_4)_2$ (4) 37.5% $Ca(H_2PO_4)_2$ (5)50% $Ca(H_2PO_4)_2$ (6) 62.5% $Ca(H_2PO_4)_2$.

Table 1. XRD results of MPBC after setting for 24 h and degraded for 30 d. (1) 0 wt% (2)12.5 wt% (3)25 wt% (4)37.5 wt%(5)50 wt% (6)62.5 wt%

	1	2	3	4	5	6
Setting for 24 h	①②	②③④⑤	②④⑤⑥⑦⑧	②④⑤⑥⑦⑧⑨	⑤⑥⑦⑧⑨	⑤⑥⑦⑧⑨
Degraded for 30 d	❷❺❻❼	❶❸❸❾	❶❷❸❿⓫	❶❷⓬	❶❷❽	❶❷❽

①MgO②KMgPO₄③CaKPO₄④Ca₃(PO₄)₂⑤Mg₃(PO₄)₂⑥MgHPO₄⑦MgCa₂ (PO₄)₂⑧HA.

⑨MgKH(PO₄)₂.

❶MgHPO₄❷Mg₃(PO₄)₂❸KMgPO₄ ❺MgH₂P₂O₇❻Mg₂P₂O₇❼K₄P₂O₇ ❽K₂CaP₂O₇❾CaKPO₄❿Mg₂KH(PO₄)₂⓫Ca₃(PO₄)₂⓬K₂CaH₄(P₂O₇)₂.

Table 2. RGR and toxicity grade conversion

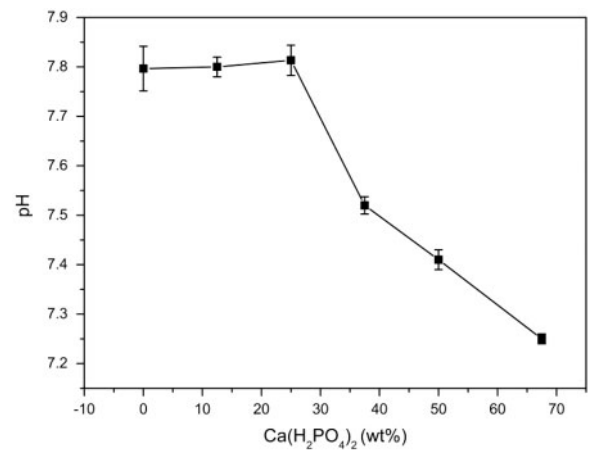| Toxicity Grade	RGR (%)
Grade 0	$\geq$100
Grade 1	75-99
Grade 2	50-74
Grade 3	25-49
Grade 4	1-24
Grade 5	0

Figure 5. Effect of $Ca(H_2PO_4)_2$ on the degradation rate.

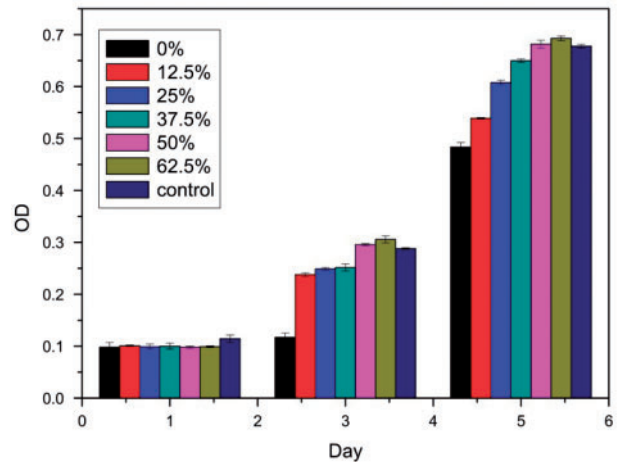**Figure 6.** results of cytotoxicity of MPBC samples.

Measurement of setting time and compressive strength

The effect of $Ca(H_2PO_4)_2$ on the setting time is shown in Fig. 2. The setting time of MPBC increased with an increase content of $Ca(H_2PO_4)_2$. When the ratio of MgO and KH_2PO_4 was 1:3, the content of MgO was excessive to the reaction (Table 2). While with the increase of $Ca(H_2PO_4)_2$, the acidity of the reaction system was increased, producing more crystalline products, and the main product $KMgPO_4$ disappeared, which extended the time to reach the equilibrium of the reaction, leading longer setting time. Figure 3 shows the effect of $Ca(H_2PO_4)_2$ on the compressive strength. The compressive strength increased with the increase of $Ca(H_2PO_4)_2$, and reached a maximum value of 31.2Mpa after setting for 24h without additional pressure, higher than the traditional NH_4-MPC, 29Mpa with added pressure [26]. The increasing compressive

strength was owing to the increase of acidity in the system, resulting in more clay-like substances, and they stacked together to form the strength (Fig. 4). Yet the decreasing of the compressive strength was because that the degree of crystallinity about the products was reduced, and the structure also became looser (Fig. 4), resulted in decreasing of compressive strength.

XRD analysis and degradation rate

The phase composition of the hardened MPBC with different content of $Ca(H_2PO_4)_2$ was characterized by XRD as shown in Table 1. The MPBC contained a mixture of $KMgPO_4$ and unreacted MgO when the raw materials were KH_2PO_4 and MgO. With the increase of $Ca(H_2PO_4)_2$, MgO disappeared, and $MgHPO_4$, $MgCa_2(PO_4)_2$,
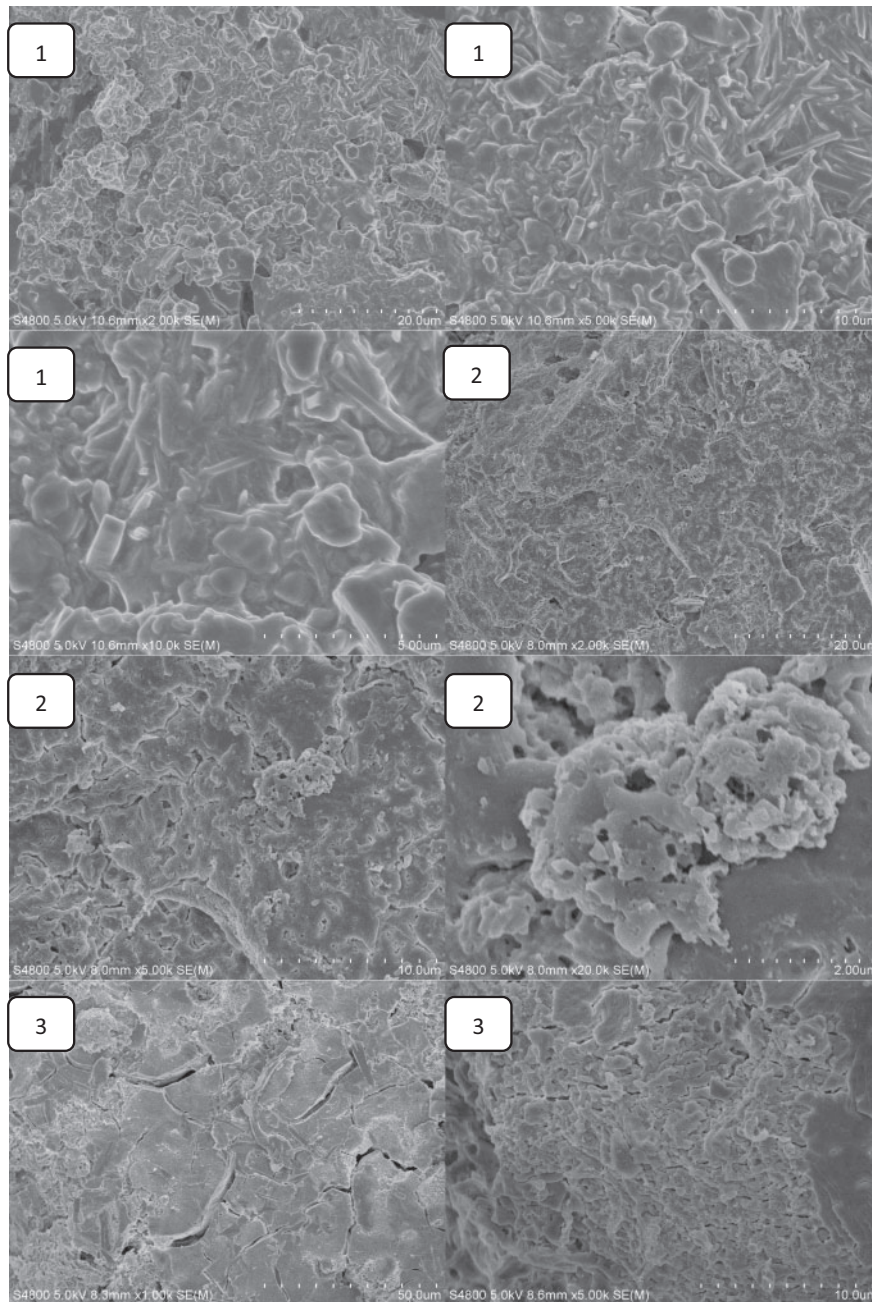

Figure 7. Effect of $Ca(H_2PO_4)_2$ on the pH in MPBC extracts.
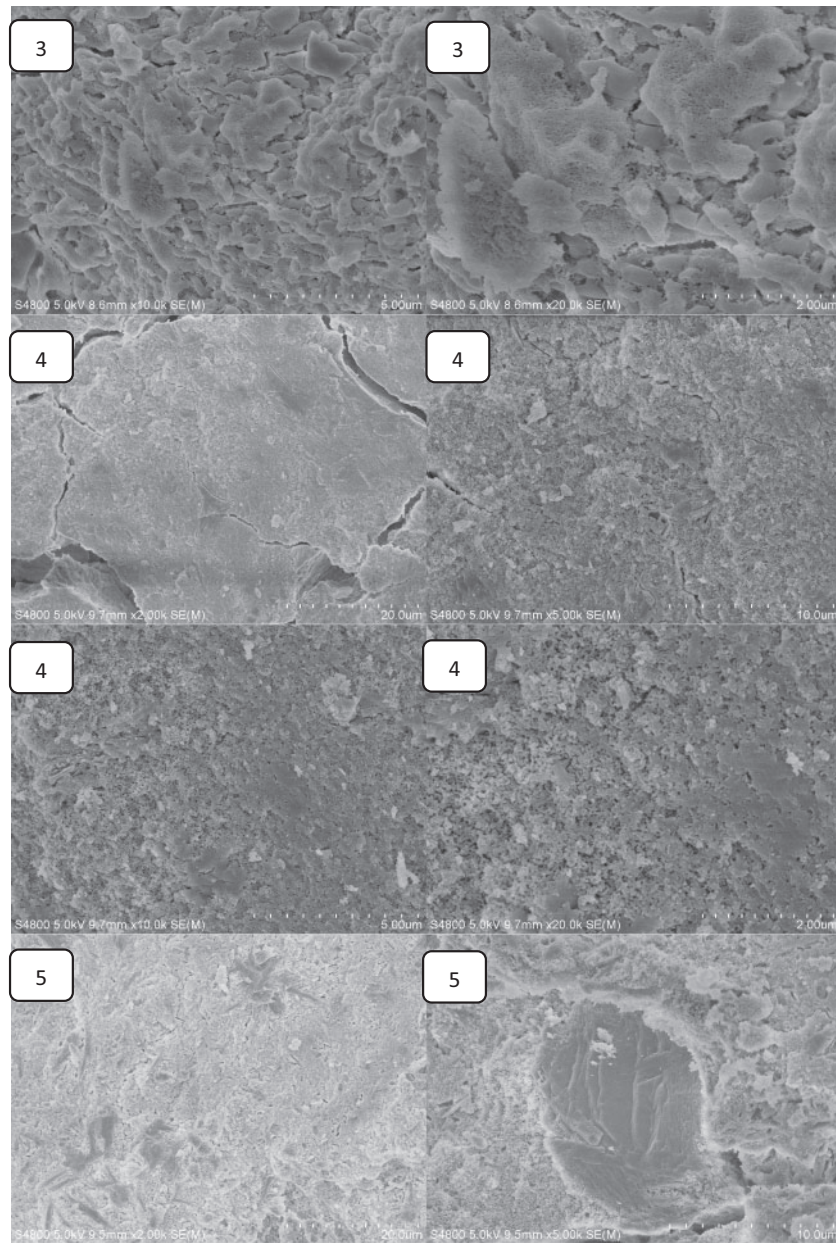
Figure 7. Continued.

Mg$_3$(PO$_4$)$_2$ and Ca$_3$(PO$_4$)$_2$ emerged. If the Ca(H$_2$PO$_4$)$_2$ was more than 50 wt%, KMgPO$_4$ would disappear, and the final composition was MgHPO$_4$, Mg$_3$(PO$_4$)$_2$, MgCa$_2$(PO$_4$)$_2$ and MgKH(PO$_4$)$_2$, which was in accordance with the results of the pH in MPBC extracts.

Figure 3 presents the weight loss ratio of MPBC samples immersed in Tris-HCl solution at different time. Clearly, the MPBC degraded in Tris-HCl solution with time, and the degradation rate was related to the amount of products as shown in Table 2. From Table 2, we could see that the amount of products after degradation for 30 days decreased the most was the MPBC sample with 37.5 wt% Ca(H$_2$PO$_4$)$_2$. And it had the fastest degradation rate, which meant the more the products disappeared, the fast the degradation rate was.

After the degradation of 30 d, the MgO and KMgPO$_4$ changed into magnesium phosphate (Mg$_3$(PO$_4$)$_2$), magnesium dihydrogen pyrophosphate (MgH$_2$P$_2$O$_7$), magnesium pyrophosphate (Mg$_2$P$_2$O$_7$) and potassium pyrophosphate (K$_4$P$_2$O$_7$). The MPBC samples with cement powder containing Ca(H$_2$PO$_4$)$_2$ would produce magnesium hydrogen phosphate (MgHPO$_4$) after setting for 24 h (Table 1). And after the degradation of 30 d, there were also MgHPO$_4$ emerged. While the other products changed into pyrophosphate.

The pH of MPBC extracts

The result of pH in MPBC extracts is shown in Fig. 5. The pH decreased with the increased content of Ca(H$_2$PO$_4$)$_2$, related to the products after setting for 24 h. With the increase of Ca(H$_2$PO$_4$)$_2$, both KMgPO$_4$ and MgO disappeared, and MgHPO$_4$ emerged, causing the decrease in pH of MPBC extracts, which could also prove the alkali of KMgPO$_4$.
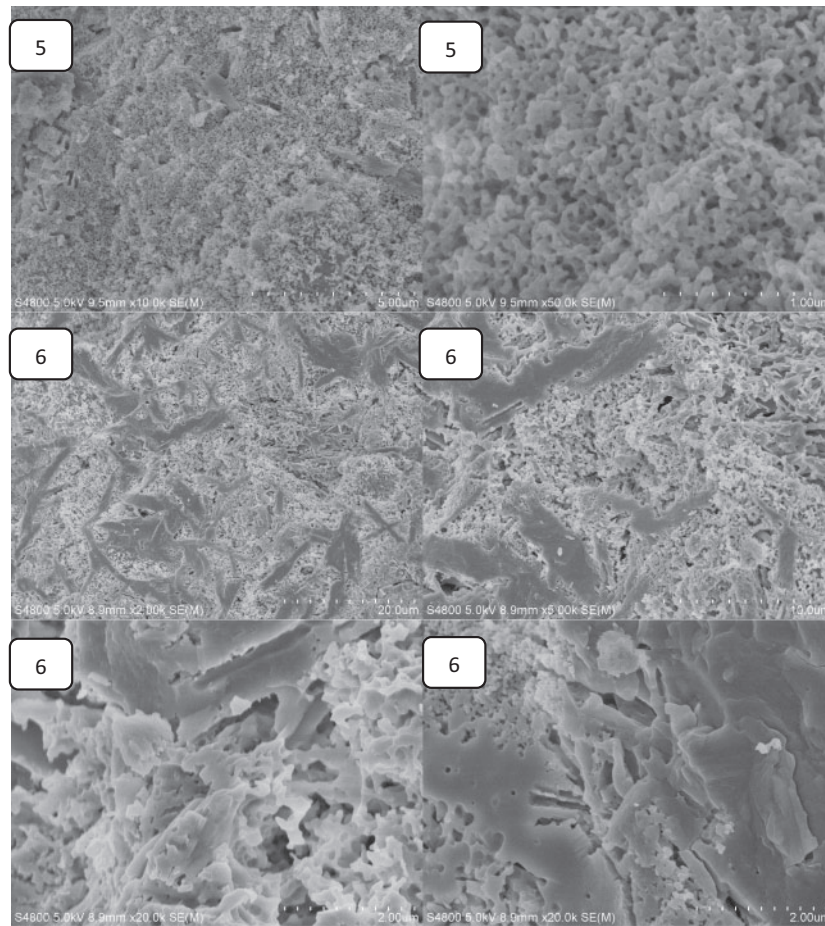
Figure 7. Continued.

The SEM images of MPBC samples

Figure 4 presents SEM images of the surface morphology/microstructure of MPBC with different content of $Ca(H_2PO_4)_2$ after setting for 24 h. It was found that the MPBC without $Ca(H_2PO_4)_2$ contained cylinder-like crystals which should be the $KMgPO_4$ and clay-like substances to form a dense structure, and the crystals were in close proximity to the clay-like substances as shown in Fig. 4(1). With the increase of $Ca(H_2PO_4)_2$, the cylinder-like crystals disappeared while the clay-like substances grown, which was corresponding to the XRD results that the $KMgPO_4$ would disappear with the increase of $Ca(H_2PO_4)_2$. And we could see that the clay-like substances stacked together to form the strength of the sample from Fig. 4(2) to Fig. 4(4). And also there were many small voids on the clay-like substances. When the content of $Ca(H_2PO_4)_2$ was more than 50%, the clay-like substances were grown to smooth surface with loose and porous structure under it, which caused the decrease of the MPBC sample.

The cytotoxicity of MPBC

The cytotoxicity of the MPBC was investigated through MTT assay. The MTT assay is very often used to evaluate cell proliferation and the viability for biomaterial toxicity. In this experiment, the extracts of the MPBC samples were adjusted to examine the cytotoxicity on L929 cells.

According to RGR and toxicity grade conversion table (Table 1), the toxicity of MPBC samples when the content of $Ca(H_2PO_4)_2$ was less than 50% were classified as grade 1, and the other samples were classified as grade 0, suggesting that they have good biocompatibility for cellular application (Fig. 6). It was because that the addition of $Ca(H_2PO_4)_2$ reduced the pH in MPBC extracts (Fig. 7), which could also prove that the alkaline $KMgPO_4$ had some bad effects on the biocompatibility.

Conclusion

A novel of degradable MPBC was developed by using a mixture of MgO, KH_2PO_4 and $Ca(H_2PO_4)_2.H_2O$ as cement powders. With the increase of $Ca(H_2PO_4)_2$, the setting time extended, and the range was 6 min to 18 min. While the compressive strength increased first and then decreased, and the maximum value could reach to 31.2 Mpa after setting for 24 h. The MPBC was degradable in Tris-HCl, and the cytotoxicity assays of MPBC extracts showed the good biocompatibility of the samples, suggesting the promise of the MPBC as a bioactive biomaterial for bone regeneration.

Acknowledgements

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