Preparation and characterization of bioactive and biodegradable Wollastonite/poly(D,L-lactic acid) composite scaffolds

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Composite scaffolds of poly(D,L-lactic acid) (PDLLA) with bioactive wollastonite were fabricated by the conventional solvent casting-particulate leaching method. The pore structures and morphology of the scaffolds were determined by scanning electron microscopy (SEM). The bioactivity of the composites was evaluated by soaking in a simulated body fluid (SBF), and the formation of the hydroxyapatite (HAp) layer was determined by SEM and energy-dispersive spectrometer. The results showed that the wollastonite/PDLLA composites were bioactive as it induced the formation of HAp on the surface of the composite scaffolds after soaking in SBF for seven days. In addition, pH and ion concentration changes of SBF solutions with composite scaffolds were examined. The results showed that the composites could release Ca and Si ions, which could neutralize the acidic degradation by-products of the PDLLA, and stabilize the pH of the SBF solutions between 6.7 and 7.2 within a three-week soaking period. Furthermore, the measurements of the water contact angles suggested that incorporation of wollastonite into PDLLA could improve the hydrophilicity of the composites and the enhancement was dependent on the wollastonite content. All these results suggest that incorporation of wollastonite into PDLLA might be a useful approach for the preparation of composite scaffolds for tissue repair and tissue-engineering applications.

1. Introduction

Poly(α-hydroxyesters), such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and their copolymers, have been widely used in tissue engineering [1–3]. These materials have many advantages, such as being biodegradable, biocompatible and easily processed into expected configuration. However, a number of problems has been encountered regarding the use of these polymers in tissue-engineering applications. One problem is the release of acidic degradation by-products, which can lead to inflammatory responses [4–7]. Another limitation of these biodegradable polymers is the lack of bioactivity so that the new bone tissue cannot bond on the polymer surface tightly [8].

Certain ceramic materials, such as hydroxyapatite (HAp), tricalcium phosphate (TCP) and selected compositions of silicate and phosphate glasses and glass-ceramics, actively interact with the biological environment and can chemically integrate with the surrounding bone tissue *in vivo*. These materials are therefore known as ''bioactive'' [9], and the commercially available Bioglass® and A/W glass-ceramic are typical examples of this type of bioactive materials. Wollastonite is a naturally occurring calcium silicate, which has been widely used as a filler in polymers and cement to fabricate composites with improved mechanical properties [10–12]. Recent studies have shown that wollastonite is bioactive and degradable, so that it might be used as a bioactive material in tissue repair or tissue-engineering research [13, 14]. In addition to their bioactivity, the most of these bioceramics can release alkaline ions, which may neutralize the acidic degradation by-products of the polymers. Considering the limitation of the polymers and the advantages of the bioceramics, one approach is to combine these two kinds of materials in order to obtain materials with optimized properties.

To date, a variety of three-dimensional, porous scaffolds based primarily on composites of biodegradable polymers, bioceramics and bioactive glasses, such as combinations of PLA and PGA with HAp or Bioglass® for tissue-engineering application have been investigated with varying degree of success [15–20].

In this study, our approach was to develop a novel biodegradable, bioactive, porous composite by incor-

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poration of wollastonite powders into poly(D,L-lactic acid) (PDLLA), which integrates the advantages of both the phases while minimizing known limitations associated with the parent phase. The results showed that the wollastonite/PDLLA composites possessed good *in vitro* bioactivity, pH-stabilization ability and improved hydrophilicity and were potential candidate as scaffolds for tissue engineering.

2. Materials and methods

2.1. Raw materials

PDLLA powders were obtained from the Institute of Medical Device (Shan Dong, China). Weight average molecular weight (MW) of PDLLA was 72 kDa. Wollastonite powders were prepared by chemical co-precipitation method. Briefly, continuous mixing of an aqueous solution of Na_2SiO_3 with an aqueous solution of $Ca(NO_3)_2$ at ambiance temperature was carried out overnight (molar ratio: Na_2SiO_3 : $Ca(NO_3)_2 = 1 : 1$). Then the stirring was stopped and the resulting calcium silicate suspension was filtered and washed with deionized water and ethanol. After being dried at 80 °C overnight followed by calcining at 800 °C for 2 h, the obtained wollastonite powders were characterized by X-ray diffraction (XRD; Geigerflex, Rigaku Co., Japan) and sieved to obtain particles between 98 and 154 μm (data not shown).

2.2. Preparation of wollastonite/PDLLA composite scaffolds

Wollastonite/PDLLA composite scaffolds were prepared using a solvent casting-particulate leaching method as reported previously [21]. Briefly, PDLLA powders was dissolved in chloroform with a concentration of 10% (w/v) and a certain amount of wollastonite powders was added into the solution with continuous stirring for 2 h in order to disperse the wollastonite powders uniformly. Sodium chloride (NaCl) particles sieved as porogens were then incorporated into the suspension, and the dispersion was cast into a 60 mm Teflon mold. The samples were air-dried under the fume hood for 24 h to allow the solvent to evaporate and subsequently vacuum-dried at 60 °C for 48 h to remove any remaining solvent. Immersing the samples in deionized water leached out porogens in the resulting wollastonite/salt/PDLLA composites. Samples were finally vacuum-dried to obtain the sponge-like scaffolds. The produced porous scaffolds were cut into disks with a diameter of 6 mm and stored in a desiccator under vacuum until use. For determination of hydrophilicity, wollastonite/PDLLA films were prepared by the same method, but without addition of salt particulates and salt-leaching process.

2.3. Characterization of composite scaffold

2.3.1. Microstructure observation by scanning electron microscopy

Surface of the samples were coated with gold and scanning electron microscopy (SEM) examination was carried out (EPMA-8705QH2; Shimadzu, Japan) at an accelerating voltage of 20 kV to observe the microstructures, such as pore size, pore distribution and pore morphology of the scaffolds.

2.3.2. Porosity measurement

Average porosity of these samples was determined using the Archimedes' Principle described by Yang [22]. In this improved method, ethanol (density ρ_e) was used as the displacement liquid and this experiment was operated at 30 °C. A density bottle filled with ethanol was weighed (W_1). A scaffold weighed W_s was immersed into the density bottle and the air bubbles were evacuated. Then the density bottle was supplemented with ethanol to full and weighed (W_2). The scaffold saturated with ethanol was taken out of the density bottle and then the density bottle was weighed (W_3). The other parameters of the scaffolds were calculated as follows:

Volume of the scaffold pore

$$V_p = (W_2 - W_3 - W_s)/\rho_e$$

Volume of the scaffold skeleton

$$V_s = (W_1 - W_2 - W_s)/\rho_e$$

The formula to calculate the porosity (ε) was proposed as follows:

$$\varepsilon = V_p/(V_p - V_s) = (W_2 - W_3 - W_s)/(W_1 - W_3)$$

The porosities of the scaffolds were recorded in Table I. All the values presented are the average of three samples.

2.4. Scaffolds soaking in SBF

Three of composite samples from each group were immersed for 3, 7, 14 and 21 days in polyethylene bottles containing 20 ml of simulated body fluid (SBF) whose ion concentration was similar to that of extra-cellular fluid [23] at 37 °C without stirring and refreshing the SBF solution. Table II shows the ion concentrations of the SBF solution and human blood plasma. After soaking, the samples were removed from the SBF solution, gently washed with deionized water and dried at room temperature. PDLLA discs without wollastonite served as controls. SEM (JSM-6700F, Japan) and energy-dispersive spectrometer (EDS, INCA Energy, Oxford Instruments, UK) were used to monitor the formation of

TABLE I Preparation conditions and porosities for porous wollastonite/PDLLA scaffolds

Samples	Wollastonite/ PDLLA (w/w)	NaCl/PDLLA (w/w)	NaCl particle size range (μm)	Porosity (%)
1	0/100	9 : 1	98–154	95.2 ± 1.5
2	20/80	9 : 1	98–154	88.7 ± 1.7
3	40/60	9 : 1	98–154	85.5 ± 1.4

TABLE II Ion concentrations of SBF and human blood plasma

Types	Ion concentrations (mM)						
	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}
SBF	142.0	5.0	1.5	2.5	148.8	4.2	1.0
Blood plasma	142.0	5.0	1.5	2.5	103.0	27.0	1.0

HAp on the surface of the composite scaffolds. The pH values of SBF were monitored during the bioactivity study by an electrolyte-type pH meter (PHS-2C; Jingke Leici Co., Shanghai, China). The SBF solutions after soaking were collected for the determination of ion concentration changes of Ca, P and Si by inductively coupled plasma atomic emission spectroscopy (ICP–AES; Varian Co., USA).

2.5. Hydrophilicity determination

The hydrophilicity of the composite was evaluated by measuring the water contact angles of the films using the sessile drop method. The water droplet was 0.5 μl to prevent gravitational distortion of the spherical profile. Each determination was obtained by averaging the results of three measurements.

2.6. Statistic analysis

Experiments were run in triplicate per sample. All data were expressed as means ± standard deviation (SD) for $n = 3$, and the student t-test was used for statistical analysis.

3. Results

3.1. Characterization of wollastonite/PDLLA composite scaffolds

3.1.1. Microstructure analysis by SEM

Fig. 1 shows SEM micrographs of the cross section of composite scaffolds subjected to varying wollastonite content. The pure PDLLA scaffold exhibited macroporous structure with interconnected open pores, and pore size varied from several tens of microns to hundreds of microns (Fig. 1(a)). After compounding with 20 wt % wollastonite, the macroporous structure was still maintained. However, compared with pure PDLLA scaffolds, some wollastonite particles are dispersed on the surface of pores, as shown in Fig. 1(b). When the wollastonite content increased to 40 wt % of the composites, more wollastonite particles were apparent on the pore surface and some particles aggregated although the macroporous structure were still maintained (Fig. 1(c)).

3.1.2. Porosity analysis

The effect of the incorporation of wollastonite on the porosity of the composites is shown in Table I. It is clear to see that the addition of wollastonite resulted in a decrease of porosity. The porosities decreased from 95 to 85% as the wollastonite content increased from 0 to 40%.

3.2. Scaffolds soaking in SBF

3.2.1. HAp formation on the composite scaffolds

The responses of wollastonite/PDLLA composites in contact with SBF were analyzed using SEM and EDS. Fig. 2 shows the SEM images of a composite with 40 wt % wollastonite before and after soaking in SBF for seven days. Before soaking, the top surface of the composites was even and many wollastonite powders aggregated in the pores (Fig. 2(a) and (b)). After soaking, it was obvious that the morphology of the scaffold surfaces was changed and some deposits were evident on the surface of the composites and the pores were still visible, as shown in Fig. 2(c). A higher magnification (Fig. 2(d)) showed that the deposits were composed of

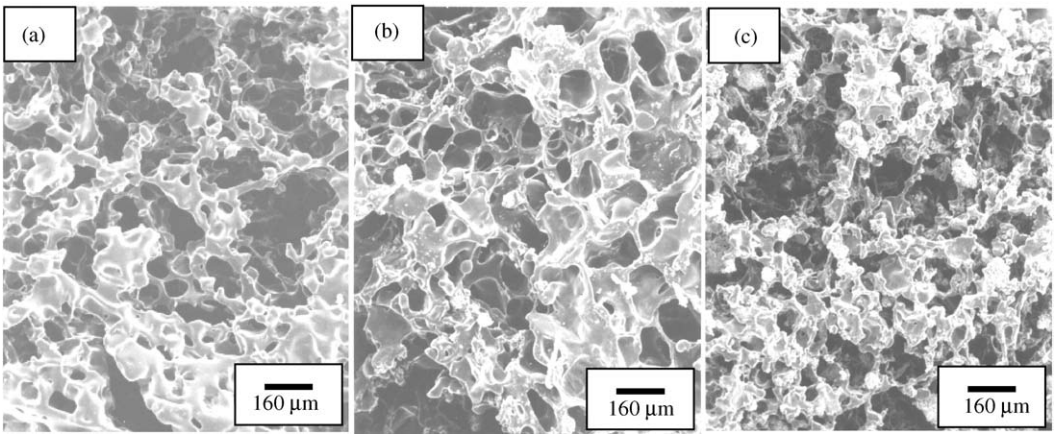

Figure 1 SEM micrographs of the scaffolds: (a) pure PDLLA scaffolds; (b) composite scaffolds, with 20 wt % wollastonite and (c) composite scaffolds with 40 wt % wollastonite.

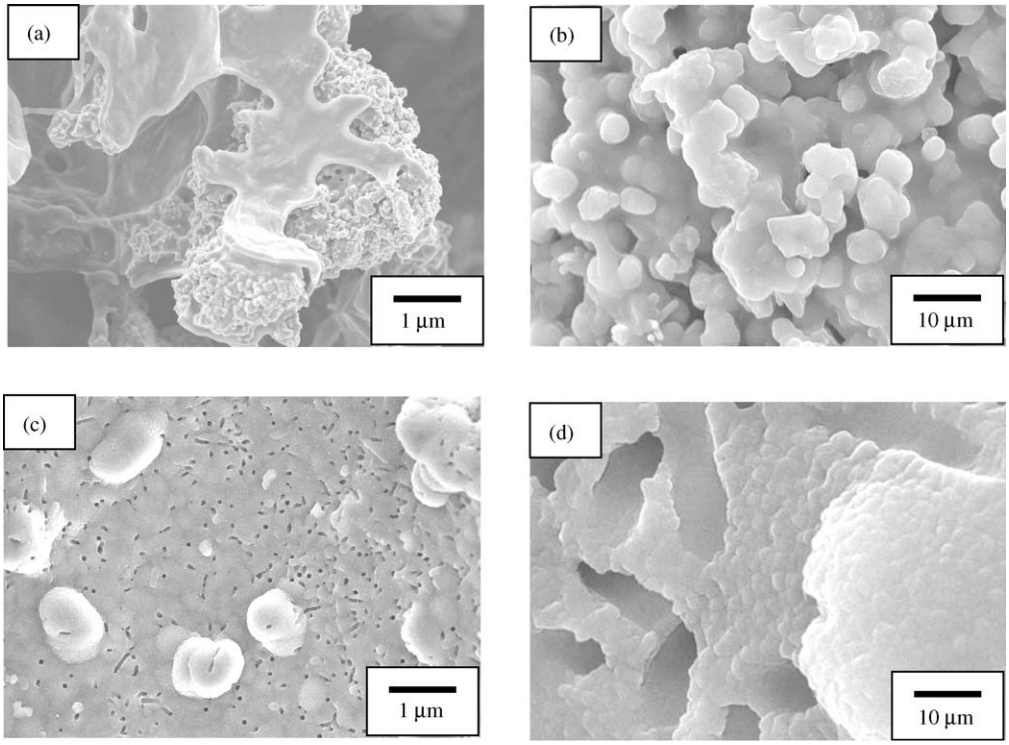

Figure 2 SEM micrographs of the composite scaffolds with 40 wt % wollastonite before and after immersion in SBF for seven days. (a) and (b) Images of composites before immersion. (c) and (d) Images of composites after immersion.

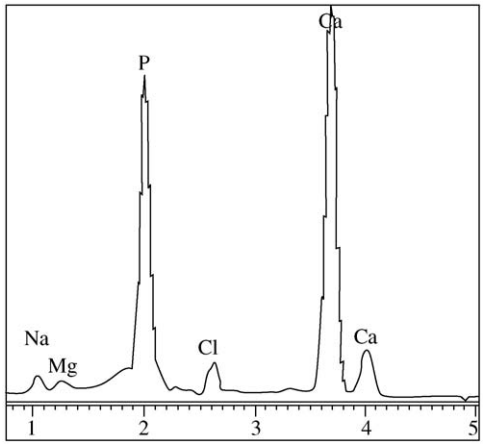

Figure 3 EDS spectra of the composite scaffolds with 40 wt % wollastonite after immersed in SBF for seven days. Note the presence of Ca, P on the surface, Ca/P = 1.61.

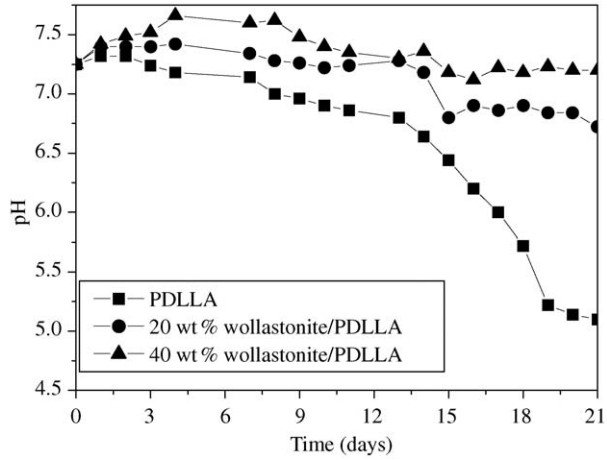

Figure 4 Changes of pH values in the SBF solution after soaking the scaffolds for various periods.

crystals with typical morphology of HAp [24], and the size of the crystals was 100–200 nm in length.

Fig. 3 shows the EDS spectra of the crystals formed on the surface of the composite with 40 wt % wollastonite after soaking in SBF for seven days. The Ca and P peaks were detected and the atom ratio between Ca and P was 1.61, which was close to the ratio for the HAp. No HAp crystals were developed on the surface of the pure PDLLA after soaking in SBF for seven days (data not shown).

3.2.2. pH and ion concentration changes of the SBF solution

Fig. 4 shows pH changes in SBF solutions after samples were soaked for various periods. For pure PDLLA scaffolds, a two-phase profile of pH changes was evident. The phase one was characterized by a slow decrease in the pH from 7.25 to 6.8 during the first 14 days, and was followed by phase two, in which the pH values showed a sharp decrease from 6.8 to 5.1 in the last seven days of soaking. For the composite samples, the pH values of the SBF solution showed slight increase in the first 4 days of soaking, and then gradually decreased in a slow rate toward the end of the soaking period. The pH for the composites with 20 wt % wollastonite was stabilized between 6.7 and 7.25, and the one for composites with 40 wt % wollastonite was between 7.1 and 7.6 during the whole soaking period.

Fig. 5(a) and (b) show changes in ion concentrations of Ca, P and Si of the SBF solutions after soaking. It was obvious that the Ca and Si ion concentrations increased rapidly within the first three days of soaking, and then continued to increase at a slower rate up to 21 days. In

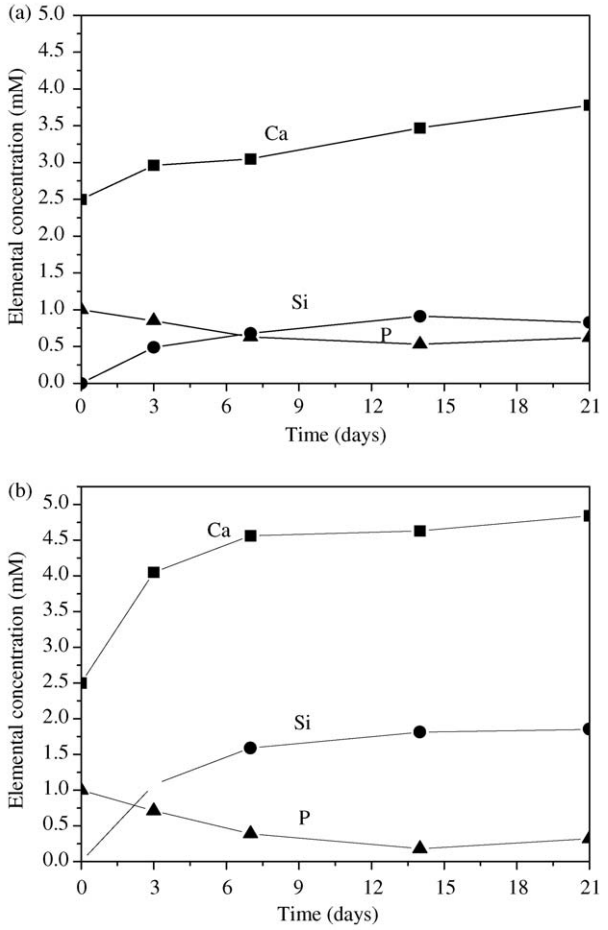

Figure 5 Changes of Ca, Si and P concentrations of the SBF solution after soaking the composite scaffolds for various periods: (a) SBF containing composites with 20 wt % wollastonite, and (b) SBF containing composites with 40 wt % wollastonite.

addition, the composites with higher wollastonite content (40%) showed a more intensive release of both Ca and Si ions as compared with the composites with lower wollastonite content (20%). In contrast to the increase in the Ca and Si concentration, P concentration of the SBF decreased gradually through the whole soaking period, and the decrease in P concentration for the composite with 40% wollastonite was more intensive as compared to the composite with 20% wollastonite.

3.3. Hydrophilicity determination

Table III shows the results of the measurements of water contact angles of the sample surfaces. It could be seen that the water contact angle of the samples was significantly reduced from 67° (pure PDLLA) to 39° (composites with 40 wt % wollastonite) by the addition of wollastonite ($p < 0.01$), which suggested an improvement in hydrophilicity.

TABLE III Water contact angles of samples

Samples	Water contact angles (degrees)
Wollastonite	0
PDLLA	67 ± 1.5
Wollastonite /PDLLA (20/80)	44 ± 1.5
Wollastonite /PDLLA (40/60)	39 ± 1.0

4. Discussion

In tissue engineering, the polymer scaffolds provide a suitable space in which seeded cells can grow and new tissues can be formed. The rates of growth of seeded cells and the formation of the new tissue are dependent on the porosity, pore diameter, pore shape and porous structure of the scaffold [2, 25, 26]. Higher porosity is required to offer sufficient space for tissue growth and to increase the volume of the invasion of surrounding tissue. An interconnecting pore network is also essential for tissue ingrowth, vascularization and diffusion of nutrients. In our study, the majority of the pore size was between 50 and 160 μm, which was in accord with the size of the salt particles. In addition, some pores larger than that of the salt particles were apparent because of the aggregation of porogens, and some were smaller as compared with the salt particles because of shrinkage of the scaffolds. However, there were some pores with the size between 10 and 20 μm dispersed on the walls of the scaffolds, which was introduced by the solvent evaporation. These pores might play an important role in the diffusion of nutrients. The porosity was affected by the addition of wollastonite, which could be seen from the Table I. With same salt content, the porosity of the pure PDLLA was higher than that of the wollastonite/PDLLA composites. As the porosity can be described as $\varepsilon = 1/(1 + V_s/V_p)$ according to the Archimedes' Principle, it is obvious that the decrease in the volume of the scaffold pore (V_p) and the increase in the volume of the scaffold skeleton (V_s) caused by the dispersion of the wollastonite powders in the scaffolds and on the wall of the pores will result in the decrease of porosity. However, our results showed that a 85% porosity of the composites with the addition of 40 wt % wollastonite can be achieved, which could still be suitable for tissue engineering [27].

Currently, bioactivity has been deemed a critical factor in facilitating the chemical fixation of biomaterials to bone tissue, and ultimately the *in vivo* success of the bone grafting materials [28–30]. Several groups have begun to explore the potential of combining bioactive glass or ceramics with PLA (or copolymers) to form composite materials for bone tissue engineering. Roether *et al*. [16] had reported a novel bioresorbable and bioactive materials based on PDLLA and Bioglass® for tissue engineering. Their result showed that the composite were bioactive and small HAp crystals were deposited on the surface of the materials after seven days of immersion in SBF. After 21 days soaking in SBF, a HAp layer was formed with a thickness of 10 μm. Ma *et al*. [31] reported that the thickness of the HAp layer on their HA-containing composites was 1 μm after 21 days of soaking in SBF. In our study, wollastonite, as a bioactive and degradable ceramic, was compounded into PDLLA to form novel composites. The composites showed high bioactivity as a HAp layer 10 μm appeared on the composite surface after seven days soaking in SBF. The EDS quantitative analysis of this layer on the composites gave a Ca/P ratio of 1.61, which was close to the Ca/P ratio for HAp.

Another significant advantage of the composite over the pure PDLLA is that the acidic degradation by-products of the pure PDLLA could be neutralized by the basic ions released from wollastonite due to its dissolution in the SBF solution. Through hydrolysis

reactions, PDLLA degrades to lactic acid when exposed to an aqueous environment, which can cause a biologically significant decrease in local pH and may lead to undesirable responses [1]. Solving the problem of controlling pH shifts would improve the biocompatibility of a variety of implantable devices for both short-term and long-term clinical use. In previous studies, many experiments have been carried out to control the pH decrease. Agrawal *et al*. [32] had studied the technique to control pH in the vicinity of biodegrading PLA–PGA implants by adding basic compounds into the polymers. The results showed that three different basic compounds, calcium carbonate (CC), sodium bicarbonate (SBC), and HAp, were effective in controlling the pH decrease. The HAp specimens exhibited an almost linear decrease in their media pH from 7.0 to 4.3 at a rate of 0.042 per day. The SBC specimens showed a precipitous decrease from 7.0 to 4.5 between five and seven weeks, and marked swelling of the implants containing CC or SBC was observed as compared with the control implants. Heidemann *et al*. [33] mixed water-soluble sodiumhydrogenphosphate (NaP) with pre-degraded PDLLA to gain pH-stabilization. The results showed that the pH of Ringer's solution containing PDLLA + NaP samples decreased from 7.4 to 5.0 at the end of a three-week incubation. Van der Meer *et al*. [34] investigated the pH changes of the composite of PDLLA and 30 wt % HAp, and the results showed that the pH dropped from 7.4 to 3.8 within three weeks. In this present study, the pH of the SBF solution containing the wollastonite/PDLLA composites was stabilized between 6.7 and 7.2 within a three-week soaking period, and the stabilization effect was dependent on the amount of wollastonite.

The ion concentration changes in the SBF solution can possibly explain the pH-stabilization ability of the wollastonite. As Kokubo [35] has proposed in the study of the mechanism of the HAp formation on A/W glass-ceramics, the first step of the reaction is that the release of Ca and Si ions from the glass-ceramics, which will form basic hydrates. Our study showed that the wollastonite/PDLLA composites could also release Ca and Si ions, which were able to form basic hydrates and neutralize the acidic degradation by-products of the PDLLA, so that the negative effect of the degradation by-products of PDLLA could be eliminated. Lu *et al*. [1] had indicated that the 45S5 bioactive glass could release alkaline ions to neutralize the acidic degradation by-products of the polymers, but there was no detailed description on the pH and ion concentration changes of the SBF in his study. In our work, when the composites were soaked in SBF, the Ca and Si ion concentrations of the SBF increased with time during the soaking period. The Ca and Si ion concentrations of the SBF solution containing composites with 40 wt % wollastonite increased more quickly than that of the SBF solution containing composites with 20 wt % wollastonite. This result indicated that higher wollastonite content could have a higher ion release rate, which resulted in higher basic ion concentrations of the SBF solution. Our results suggested that wollastonite was effective in neutralizing the acidic by-products of PDLLA, and the stabilization ability of the composites could be controlled by adjusting the amount of wollastonite in the composites.

Besides bioactivity, and pH-stabilization ability, the surface properties of a biomaterial would greatly affect the performance of a biomaterial in a biological environment. PDLLA has been widely used in a variety of clinical applications and in tissue-engineering research [36]. However, the lack of tissue compatibility and resistance to biological environment were the problems that still remained [37]. Therefore, how to improve the interactions between biomaterials and cells for eliciting controlled cellular adhesion and maintaining differentiated phenotypic expression had become one of the challenges in the field of tissue engineering [38]. As for PDLLA, it was difficult to be modified since there were no reactive, functional groups on its surface. Therefore, exploring different methods would benefit the development of tissue engineering. Cai *et al*. [39] had used silk fibroin (SF) to modify the PDLLA films. The results suggested that SF positively affected the growth and differentiated function of osteoblasts. In addition, they investigated the possibility to modify PDLLA using poly(aspartic acid) (PASP) [40]. The results showed that the PASP was immobilized on the surface of PDLLA film and the surface hydrophilicity of the PDLLA films was improved. Chim *et al*. [41] used gas plasma to treat the surface of 3-D PLA scaffolds and the results showed that this method enhanced the cell adhesion, proliferation and differentiation over 10 days in culture using human embryonic palatal mesenchyme cells. Another approach was to combine hydrophilic inorganic materials with polymer in order to improve the surface characteristic of the materials. In our study, we investigated the hydrophilicity of the wollastonite/PDLLA composite by measuring the water contact angle. Our results showed that the water contact angles of the materials decreased from 67° to 39° as the wollastonite content in the composites increased from 0 to 40 wt %, which suggested a remarkable improvement of the hydrophilicity of the composite by incorporation of the wollastonite..

5. Conclusion

Three-dimensional, porous, wollastonite/PDLLA composite scaffolds were prepared by a solvent casting-particulate leaching method. These scaffolds were bioactive, confirmed by the formation of the HAp layer on the surface of the composites after immersing in SBF for seven days. In addition, the composite scaffolds showed the ability to compensate the pH decrease caused by the acidic degradation by-products of the PDLLA, and the pH of the SBF solution could be maintained in the physiological range during a three-week soaking experiment. Furthermore, the hydrophilicity of the pure PDLLA was improved by adding wollastonite, and this improved property, together with the bioactivity and pH compensation ability, make these scaffolds potential candidates for tissue repair and tissue-engineering applications.

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