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Fabrication of fibrous poly(butylene succinate)/wollastonite/apatite composite scaffolds by electrospinning and biomimetic process

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Abstract In this paper, a novel kind of Poly(butylene succinate) (PBSU) /wollastonite/apatite composite scaffold was fabricated via electrospinning and biomimetic process. Pure PBSU scaffold and composite scaffolds with 12.5 wt% and 25 wt% wollastonite were firstly fabricated by electrospinning. SEM micrographs showed that all the electrospun scaffolds had homogeneous fibrous structures with interconnected pores and randomly oriented ultrafine fibers. The composite scaffolds were then surface modified using a biomimetic process. SEM and XRD results showed that apatite could deposit on the surfaces of the composite fibers after incubation in SBF and a novel fibrous structure with microspheres composed of worm-like apatite on composite fibers was formed. Incubation time and wollastonite content were found to influence the morphology of the scaffolds during the biomimetic process obviously. Both the amount and the size of the microspheres on the composite scaffolds increased with increased incubation time. After a certain incubation time, microspheres formed on the composite fibers with less wollastonite had a relatively larger size. Therefore, the microstructure of the composite scaffolds could be adjusted by controlling the wollastonite content and the incubation time. All of these results suggest that it is an effective approach to fabricate PBSU/wollastonite/apatite fibrous composite scaffolds with different material content and controllable microstructure for bone tissue engineering.

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Introduction

Bone fractures caused by trauma are serious health problems and none of the currently used solutions is perfect. Tissue engineering techniques, introduced by Langer and Vacanti in 1993 [1], offer a promising approach to bone repair and regeneration [2–4]. In this approach, biomaterials play an important role by serving as scaffolds for cell attachment, growth and tissue regeneration [5–8].

Electrospinning is an economical and simple approach capable of providing ultrafine fibers with extremely long length and high specific surface area [9, 10]. With these special fine properties, electrospinning has been employed as a new approach for preparing suitable fibrous structures for tissue engineering applications [11–15]. Electrospun scaffolds have fibrous structures, which are similar to that of the natural extracellular matrix (ECM). In addition, fibrous scaffolds fabricated by electrospinning have many other advantages, including the high porosity, interconnected pores and relatively large surface areas. All of these structural characteristics promote favorable responses of seeded cells in vitro, such as enhanced cell attachment and proliferation [11, 14, 15].

While used in tissue engineering, the materials should be biodegradable and be able to promote cell adhesion and activity [16]. Various materials have been investigated as biomaterials used in bone tissue engineering, such as ceramics, bioactive glasses and polymers [17–21]. As a novel biomaterial, poly(butylene succinate) (PBSU) has

been demonstrated to possess good biocompatibility and adequate biodegradability [22]. However, some limitations have been encountered considering the use of PBSU in bone tissue engineering applications, such as the lack of bioactivity.

Therefore, a promising approach for preparation of composite scaffolds comprising a biodegradable polymeric phase and a bioactive ceramic phase was proposed to solve these problems. As a naturally occurring calcium silicate, wollastonite has been demonstrated to be bioactive and degradable. In the previous work of our group, Li has fabricated composite scaffolds of wollastonite/poly (D,L-lactic acid) and wollastonite/PHBV [18, 19], and showed that the addition of wollastonite to the biodegradable polymers resulted in composite scaffolds with good bioactivity, which was confirmed by the formation of the HAp layers on the surface of the composites after soaking in SBF for several days. Thus, it is supposed that the incorporation of wollastonite into PBSU is a useful approach to obtain composite scaffolds with improved properties.

In order to obtain a better support for bone cell adhesion, proliferation and differentiation, modification of the surface of the composite scaffolds is considered. As one of the primary components of extracellular bone matrix, apatite has been demonstrated to possess good osteoconductivity [23], high affinity to living cells [24], and an ability to adsorb proteins [25]. Because of these unique advantages of apatite, surface modification by forming a apatite layer on scaffolds surface has been widely used for bone tissue engineering [26–28] and the HAp-containing scaffolds have been demonstrated to have improved osteoblastic cell seeding rate and enhanced expression levels of bone makers.

In this paper, fibrous PBSU/wollastonite composite scaffolds with different microstructures were fabricated via electrospinning and the surfaces of the scaffolds were modified by biomimetic process to form an apatite layer induced by wollastonite incorporated in PBSU.

Materials and methods

Materials

Poly(butylene succinate) (PBSU) was generously provided by the Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences (Shanghai, China). The weight-average molecular weight (M_w) of the PBSU was 3.0×10^5. The reagents used for the preparation of simulated body fluid were the followings: sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$), potassium chloride (KCl), potassium phosphate dibasic trihydrate ($K_2HPO_4 \cdot 3H_2O$), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), calcium chloride ($CaCl_2$), sodium sulfate (Na_2SO_4), hydrochloric acid (HCl), trishydroxymethylate-aminomethane [$(CH_2OH)_3CNH_2$]. All regents except PBSU used in this experiments were of analytical grade and purchased from Biomed. Co. Ltd. (Shanghai, China).

Preparation of wollastonite nanowires

Wollastonite (β-$CaSiO_3$) nanowires were prepared by a hydrothermal route as described previously [29]. The nanowires have a fiber-like shape, 50–100 nm in diameter and 10–15 μm in length as shown in Fig. 1.

The nanowires were then surface-modified using dodecyl alcohol in order to enhance the dispersion of wollastonite and promote the interfacial adhesion between the inorganic nanowires and PBSU. The procedure used in this part was based on the procedures reported previously [30, 31]. The wollastonite nanowires were dispersed in dodecyl alcohol under vigorous stirring. The resulting wollastonite-dodecyl alcohol suspensions were heat treated at 190 °C for 3 h. After aging, the wollastonite nanowires were washed in ethyl alcohol for three times and dried at 60 °C overnight for further use.

Electrospinning

In this study, three kinds of solutions were prepared for electrospinning process as shown in Table 1.

For pure PBSU solution, PBSU was dissolved in the mixture of $CHCl_3$ and C_2H_5OH directly while for the mixed solutions, the wollastonite nanowires were firstly dissolved in the mixture solvent and then ultrasonically dispersed for 30 min before the PBSU was dissolved. The concentrations of the solutions were 0.11 g/ml. The

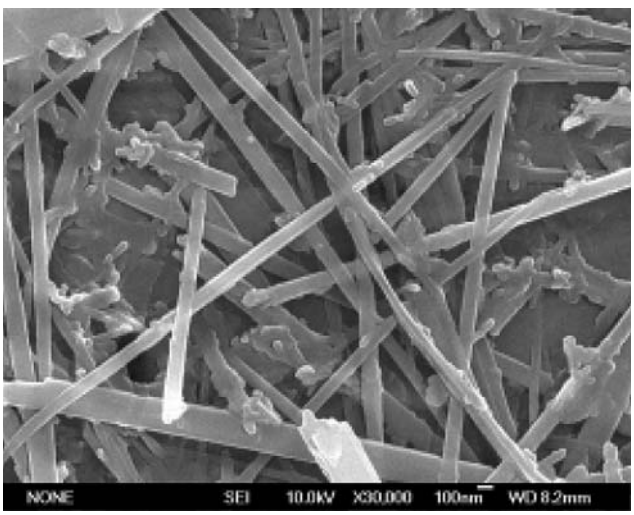

Fig. 1 SEM micrograph of wollastonite nanowires

Table 1 Different kinds of solutions prepared for electrospinning

No.	Samples	Solvent
1	Pure PBSU	75 vol% $CHCl_3$ and 25 vol% C_2H_5OH
2	87.5 wt% PBSU and 12.5 wt% wollastonite nanowires	75 vol% $CHCl_3$ and 25 vol% C_2H_5OH
3	75 wt% PBSU and 25 wt% wollastonite nanowires	75 vol% $CHCl_3$ and 25 vol% C_2H_5OH

solution was placed in a plastic syringe and supplied at a feeding rate of 1.0 ml/h using a syringe pump while electrospinning. The voltage applied to the needle of the syringe was 25 kv and the distance between the tip of the needle and the aluminum foil collector was 10 cm. The electrospinning processes were kept for 3 h respectively and the collected scaffolds were dried in the air for 24 h before SEM observation.

Morphology of electrospun scaffolds

The surface of the as-obtained scaffolds were coated with gold and their morphology was observed using a scanning electron microscope (Model JSM-6700F, Japan) at an accelerating voltage of 10 kV. The working distance of the SEM equipment was 7.9 mm or 8.0 mm.

Incubation in SBF

The SBF solution was prepared according to the procedure described by Kobubo [32] and Table 2 shows the ion concentration of the SBF solution and human blood plasma.

The specimens were cut into small pieces with dimensions of 10 × 10 mm and each specimen was immersed in a plastic bottle containing 20 ml SBF for 1, 3, and 7 days respectively. Incubation was carried out at 37 °C without stirring and the SBF solutions were renewed everyday to ensure the ion concentrations nearly equal to that of the human environment. After incubation, the specimens were washed with distilled water for three times and then dried by freeze-drying.

Characterization

The scaffolds after incubation in SBF were examined by X-ray diffraction (XRD) with a Japan Rigaku D/max 2550V X-ray diffractometer with monochromated CuKα radiation (λ = 154, 178 nm). A scan rate of 0.02 s^{-1} was applied to record the patterns in the 2θ range of 10–80°. The morphology of the incubated scaffolds was also examined using the SEM method mentioned above.

Table 2 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

Results

Morphology of PBSU and PBSU/wollastonite scaffolds

The thickness of the scaffolds was approximately 0.25 mm. The porosity of the scaffold was calculated from the ratio of the fiber mat density to the solid density [33]. Table 3 lists the densities and the calculated porosities of the scaffolds. It could be found that densities increased obviously with the increase of the wollastonite content, while the porosities keep steady to about 75%.

The SEM morphology of pure PBSU scaffold and PBSU/wollastonite composite scaffolds is shown in Fig. 2. It could be observed that all the three kinds of scaffolds have relatively homogeneous fibrous structures with interconnected pores and randomly oriented ultrafine fibers with diameters ranging from hundreds of nanometers to approximately 1 μm. However, the average diameter of the pure PBSU fibers is smaller than those of the composite fibers. The surface of the electrospun fibers is smooth and almost no wollastonite nanowires were observed on the surface of the PBSU/wollastonite composite fibers.

XRD Characterization of PBSU/wollastonite/apatite scaffolds

Figure 3 shows the XRD patterns of composite scaffolds with 12.5 wt% and 25 wt% wollastonite after incubated in SBF for 1, 3, and 7 days, respectively. All the XRD curves

Table 3 Porosities calculated from solid densities and fiber mat densities

Material	Solid density (g/cm^3)	Fiber mat density (g/cm^3)	Porosity (%)
Pure PBSU	1.140	0.282	75.3
PBSU/12.5 wt% wollastonite	1.232	0.301	75.6
PBSU/25 wt% wollastonite	1.341	0.330	75.4

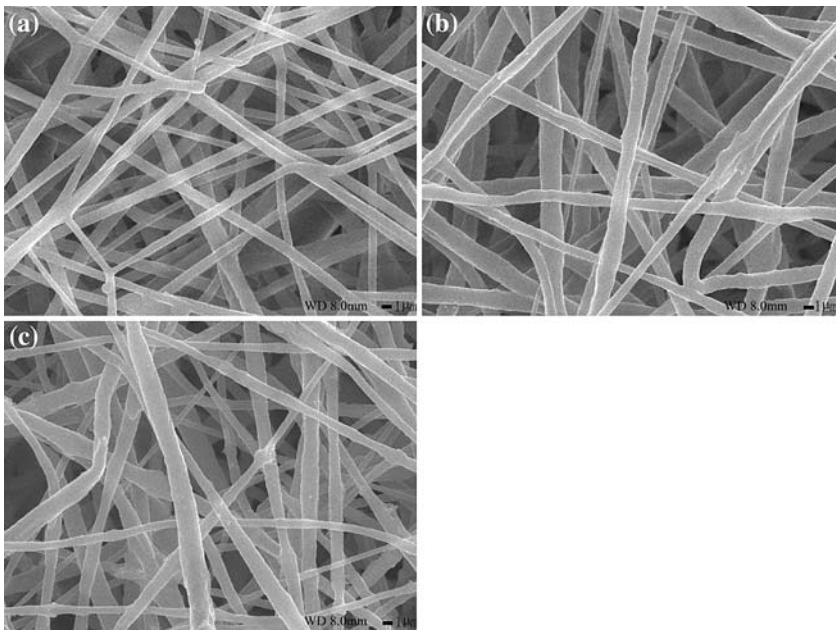

Fig. 2 SEM micrographs of electrospun scaffolds: (**a**) pure PBSU scaffold; (**b**) composite scaffold with 12.5 wt% wollastonite; (**c**) composite scaffold with 25 wt% wollastonite

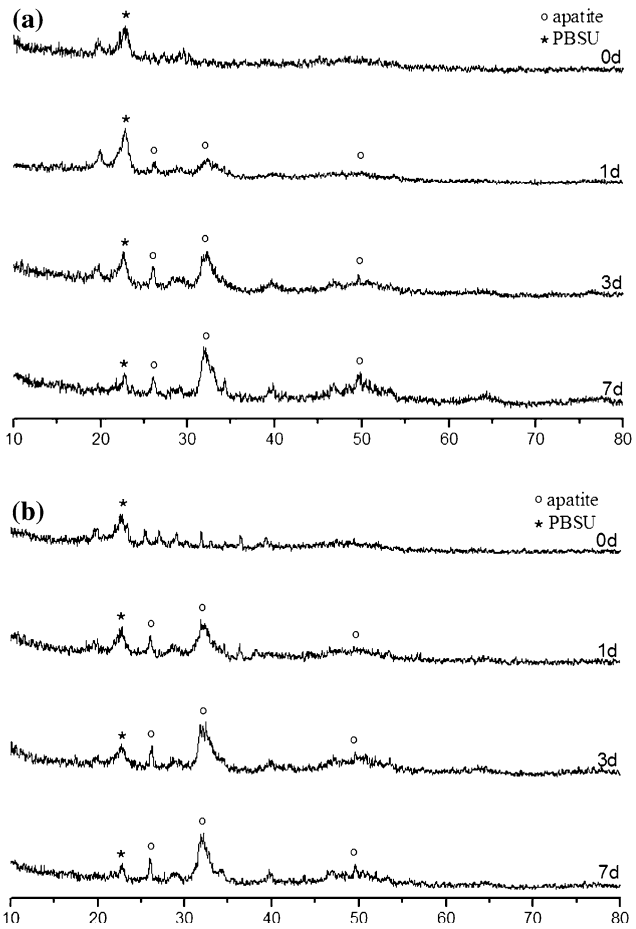

Fig. 3 XRD patterns of composite scaffolds after incubation in SBF for 1, 3, and 7 days: (**a**) composite scaffold with 12.5 wt% wollastonite; (**b**) composite scaffold with 25 wt% wollastonite

of patterns after incubation show two broad peaks at 26° and in the range of 31–33° assigned to the 002 diffraction and the 211, 112, and 300 diffractions of apatite, respectively [34]. The broad peak around 50° gives further confirmation of the presence of apatite. The peaks in the range of 22–24° in all the curves are assigned to the diffractions of PBSU. As incubation time increases, the apatite peaks become more and more obvious, while on opposite, the PBSU peaks tend to disappear.

Morphology of PBSU/wollastonite/apatite scaffolds

After incubation in SBF for 1, 3, and 7 days, no apatite was found in pure PBSU scaffold.

Figure 4 shows the SEM micrographs of composite scaffolds containing 12.5 wt% and 25 wt% wollastonite after incubation in SBF for 1, 3, and 7 days. It could be observed that a novel structure with microspheres on composite fibers was formed after incubation. The microsheres formed on the surfaces of the fibers did not cover the spaces between the fibers and after incubation the scaffolds maintained the highly porous fibrous structure. Figure 5 shows the high magnification SEM micrographs of microspheres formed on the composite fibers after incubation in SBF for 1, 3, and 7 days. It is clear to see that all the microspheres were composed of nano-sized worm-like apatite particles and no obvious difference was found on the shape of the apatite.

The amount of microspheres composed of apatite on the fibers after incubation in SBF for 3 days has increased obviously compared with that incubated for 1 day, but there is no significant difference between the amount of the

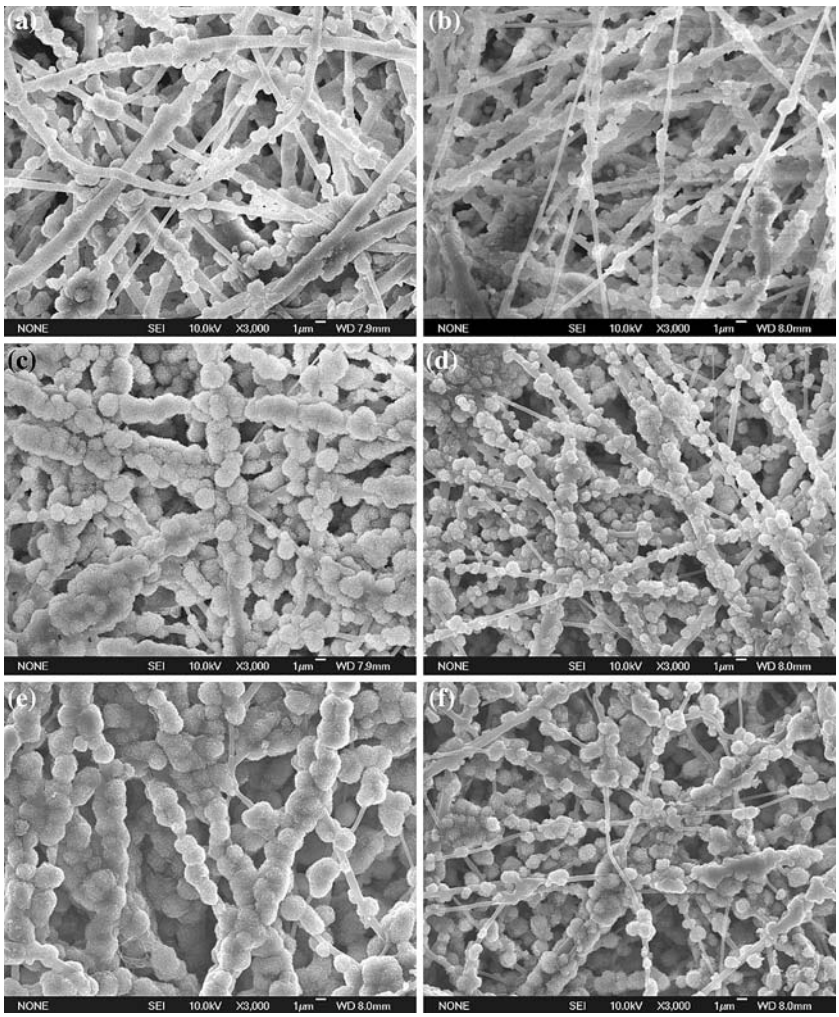

Fig. 4 SEM micrographs of composite scaffolds with 12.5 wt% wollastonite after incubation in SBF for (**a**) 1 day; (**c**) 3 days; (**e**) 7 days and composite scaffolds with 25 wt% wollastonite after incubation in SBF for (**b**) 1 day; (**d**) 3 days; (**f**) 7 days

microspheres on the fibers after incubation for 3 and 7 days. Besides the amount, significant differences between the size of the microspheres formed on the scaffolds with different amount of wollastonite and incubation time were also ovserved. While containing the same content of wollastonite, the size of the microspheres increased with the incubation time, and the sizes of the microspheres on the PBSU/12.5 wt% wollastonite composite fibers were much bigger than those on the PBSU/25 wt% wollastonite composite fibers.

Discussion

PBSU is expected to experience an extremely strong shear force during the electrospinning process, thus the nanowires suspended in PBSU solutions were forced to orientate along the axis of the composite fibers. Some wollastonite nanowires were break into shorter parts during ultrasonic dispersion and electrospinning process and wrapped by PBSU more easily. Thus, the composite fibers are smooth and almost no wollastonite nanowires could be observed on the surfaces.

It has been demonstrated that the average diameter of the electrospun fibers increases with increased viscosity of the solutions before electrospinnning [9, 10] and the addition of the wollastonite nanowires into the PBSU solutions increased the viscosity of the solutions for electrospinning. Therefore the composite fibers had a larger average diameter comparing with the pure PBSU fibers.

After surface modification of the composite scaffolds, novel fibrous structures with microspheres composed of worm-like apatite on ultrafine composite fibers were formed. To form an apatite layer on scaffold surface, biomimetic process has been widely used. However, most of the experiments were processed on the scaffolds with relatively large plane surface. Recently, Ito et al. fabricated novel composites, which composed of apatite and electrospun biodegradable PHBV nanofibers [35]. However, the apatite microspheres were formed between the fibers,

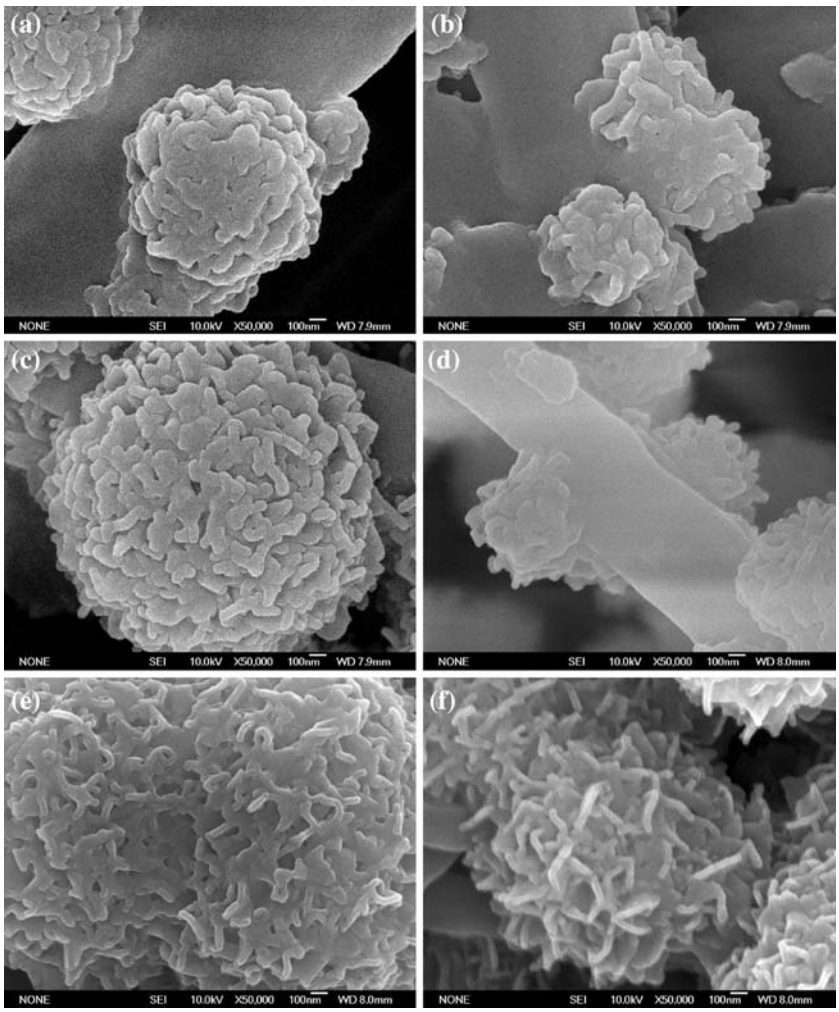

Fig. 5 SEM micrographs of microspheres composed of worm-like apatite formed on the composite fibers with 12.5 wt% wollastonite after incubation in SBF for (**a**) 1 day; (**c**) 3 days; (**e**) 7 days and on the composite fibers with 25 wt% wollastonite after incubation in SBF for (**b**) 1 day; (**d**) 3 days; (**f**) 7 days

which covered the spaces between the fibers of the fibrous scaffolds. In our experiments, wollastonite played an important role in maintaining the highly porous fibrous structures of scaffolds and the apatite layers were formed on surface the fibers. To form apatite on composite fibers, it is prerequisite that the wollastonite particles could get in contact with the SBF, by which apatite nuclei were firstly formed on the fiber surface, and then the apatite layers were gradually formed on the fibers instead of adsorbed between the fibers.

A time dependent changes of the morphology of the apatite layers on the composite fibers are obvious with the increase of the incubation time in SBF. At the beginning of the biomimetic process, with the increase of the incubation time, more and more wollastonite particles inside the fibers got in contact with the SBF. Consequently, more apatite nuclei were formed, and as a result, the amount of the microspheres increased.

The amount of the wollastonite nanowires in the composite fibers is another key parameter that influences the size of the microspheres formed on the polymer fibers and the morphology of the composite scaffolds after surface modification. It has been reported that the addition of wollastonite into polymer scaffolds could influence the degradation of the polymer significantly by changing the pH and ion concentration of the SBF solution, and the polymer degradation decreased with the increase of the wollastonite content [36]. It is possible that the polymer degradation products released into the SBF solution could affect the size and morphology of the deposited apatite. However, the influence mechanism of the polymer degradation on apatite deposition is still unclear and further investigation is required.

Therefore, the fibrous structures of the composite scaffolds could be adjusted by controlling the wollastonite content and incubation time, and promising microstructures for cell attachment and proliferation could be engendered.

Conclusions

In this experiment, pure PBSU scaffolds and composite scaffolds with different wollastonite content were firstly

fabricated by electrospinning and the electrospun scaffolds have relatively homogeneous fibrous structures with interconnected pores and randomly oriented ultrafine fibers. The composite scaffolds were then surface modified by incubation in SBF solution. After biomimetic process, a novel fibrous structure with microspheres composed of worm-like apatite on composite fibers was formed. Both the amount and the size of the microspheres were affected by the incubation time and the wollastonite content in the electrospun fibers. Thus, various PBSU/wollastonite/apatite composite scaffolds with different wollastonite content and different fibrous structure could be fabricated using electrospinning and biomimetic process. We believe that this kind of fibrous composite scaffold with controllable microstructure may have potential applications in bone regeneration and tissue engineering.

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