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Bredigite Reinforced Electrospun Nanofibers for Bone Tissue Engineering

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Abstract

In this study, bredigite nanoparticles were treated with an organosilane coupling agent to enhance its dispersability and compatibility with polymers. The polyhydroxybutyrate-co-hydroxyvaletare (PHBV) nanofibrous scaffolds containing treated bredigite (T-BR) nanoparticles were developed using electrospinning technique. It was found that pre-treating of bredigite was effective in enhancing nanoparticles dispersion both in the solution and in the PHBV matrix. Mechanical properties of the PHBV nanofibrous scaffolds were remarkably improved by incorporation of T-BR nanoparticles. The results also demonstrated that bioactivity and biodegradability rate of PHBV nanofibrous scaffolds were greatly altered by addition of BR and T-BR nanoparticles. Our study demonstrated that incorporation of T-BR nanoparticles within PHBV nanofibers may improve its mechanical performance and bioactivity, making it more appropriate materials for bone tissue engineering applications.

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Keywords: Bredigite nanoparticles; Agglomeration; Surface treatment; Nanofibrous scaffolds

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1. Introduction

Biomimetic nanofibrous structures have attracted considerable interest in bone tissue engineering applications, especially those containing polymer matrix reinforced with ceramic nanoparticles, since they have ability to mimic the structure of bone extracellular matrix [1,2]. However, ceramic nanoparticles tend to agglomerate in the polymer matrix due to the incompatible polarity of the nanoparticles with polymers which results in undesirable mechanical properties [3].There are different approaches proposed and investigated for improving the compatibility and dispersability of ceramic nanoparticles with polymers including treatment with some organic modifier such as cetyl trimethylammonium

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bromide (CTAB), polyethylene glycol (PEG), and citric acid [4,5]. Here we used glycioxypropyl-trimethoxysilane (GPTMS) as a coupling agent to improve the dispersion of the nanoparticles within polymer matrix. Bredigite ($Ca_7MgSi_4O_{16}$) is a bioceramic belonging to ternary system SiO_2-MgO-CaO and has been proved to possess superior bioactivity, biocompatibility and biological properties compared to calcium phosphate bioceramics [6, 7]. Polyhydroxybutyrate-co-(3-hydroxyvalerate) (PHBV) is a member of the polyhydroxyalkanoates family which has been extensively investigated as scaffolding materials for tissue regeneration applications, due to its biocompatibility and non-toxicity in vivo [8,9]. Among the different techniques for fabricating nanofibrous scaffolds, electrospinning is the most widely used technique, because of its ease of use and relatively inexpensive instrumental set-up [1, 8]. Moreover it is capable of incorporating nanoparticles through nanofibers. In the current study, bredigite nanoparticles were modified with GPTMS and incorporated into PHBV nanofibers using electrospinning technique. For comparison, PHBV nanofibers containing unmodified bredigite nanoparticles were also developed. Morphological and mechanical characterizations of the developed scaffolds were reported and bioactivity and biodegradation of the nanofibrous scaffolds were also evaluated in vitro.

2. Materials and methods

2.1. Surface treatment of BR nanoparticles and development of electrospun nanofibrous scaffolds

Bredigite nanoparticles obtained from our previous work [10] was mixed with 3-(glycidoxypropyl) trimethoxysilane (GPTMS, Sigma Aldrich) solution in ethanol (1:50 v/v) followed by a stirring at 60°C for 2 h, the temperature was raised up to 75 °C to evaporate ethanol and the GPTMS treated bredigite (T-BR) nanoparticles were dried for 24 h at 120 °C under vacuum.

Electrospun PHBV/BR and PHBV/TBR nanofibers were developed according to our previous study [8]. Briefly, desired amount of nanoparticles were sonicated in HFIP (1,1,1,3,3,3 hexafluoro-2-propanol, Sigma) for 30 min, then PHBV (TianAn Enmat chemical company, China) was added to the prepared mixture to obtain a total concentration of 8wt%. The electrospinning performed at the flow rate of 1 ml/h with a high voltage of 14 kV.

2.2. Characterization of nanoparticles

Fourier transform infrared (FTIR) spectra of nanoparticles was measured using Avatar 380 (ThermoQ14 Nicolet, MA), at a resolution of 4 cm^{-1} and a spectral range of 4000-400 cm^{-1} to identify the specific functional groups on the surface of the nanoparticles. Dispersion stability of obtained nanoparticles was measured by sedimentation test. Nanoparticles were dispersed in HFIP and the time required for all particles precipitating out of the solution and leaving a completely clear supernatant was recorded.

2.3. Characterization of nanofibrous scaffolds

The morphology of the nanofibrous scaffolds was study by field-emission scanning electron microscopy (FE- SEM: HITACHI S-4300, Japan). The mechanical characteristics of the scaffolds were tested using a tabletop tensile tester (Instron 5943, USA). The nanofibrous scaffolds were cut into rectangular strips of 10 ×20 mm^2 and tested at a crosshead speed of 10 mm/min with a load cell capacity of 50 N. The tensile strength measurements repeated five times for each sample.

2.4. In vitro bioactivity and biodegradation assessment

The bioactivity of the prepared composite nanofibrous scaffolds was evaluated in simulated body (SBF) solution with the composition and ionic concentration similar to those of human body plasma which was prepared according to Kokubo procedure [11]. Formation of apatite layer on the surface of the scaffolds was confirmed by inductively coupled plasma atomic emission spectroscopy (ICP-OES, Perkin-Elmer Optima 7300DV). In vitro degradation of nanofibrous web was studied in phosphate buffer saline (PBS), and the specimens were subjected to SEM to evaluate their morphological changes during degradation test.

3. Results and discussion

In our previous study, PHBV nanofibers containing different concentration of bredigite nanoparticles were developed. It was observed that at higher concentration (15%) of bredigite nanoparticles, mechanical performance of the nanofibrous scaffolds reduced, due to nanoparticles agglomeration [8]. Here, bredigite nanoparticles were modified with a silane coupling aging and incorporated in PHBV nanofiber at the concentration of 15% using electrospinning method. Figure 1 shows the spectra for bredigite nanoparticles before and after modification. Additional characteristic epoxide bond at 892 cm^{-1} and Si\OCH bonds at 1115 cm^{-1} and 1090 cm^{-1} were appeared in the spectra of T-BR nanoparticles compared to pure BR spectra, confirming the presence of GPTMS on BR surface [12]. Sedimentation test was also performed for the BR and T-BR nanoparticles in HFIP to study the dispersion stability of the nanoparticles. Both nanoparticles showed uniform dispersion immediately after the mixing. However, the sedimentation time of T-BR nanoparticles increased from 10 min to 2 h compared unmodified bredigite nanoparticles proving effective surface treatment.

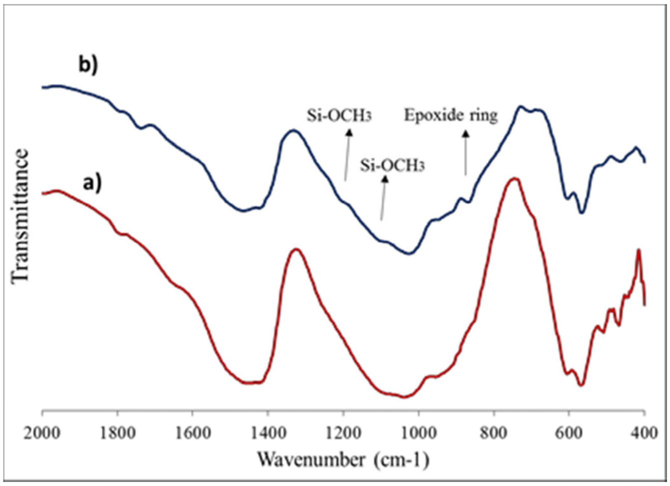

Fig. 1. FTIR spectra of a) bredigite and b) modified bredigite nanoparticles

Figure 2 shows the schematic illustration and SEM images of PHBV nanofibers containing 15% of either BR or T-BR nanoparticles. PHBV composite nanofibers containing row bredigite nanoparticles (Figure 2(a)) showed the rough morphology consisting of many more agglomerates of nanoparticles. When hydrophilic inorganic nanoparticles are combined with hydrophobic polymers, inhomogeneous dispersion of the nanoparticles in the polymer matrix will happen. Nanoparticles with enormous surface to area and high surface energy show high tendency to agglomeration in polymer matrix due to the weak interface with polymer matrix [3]. Figure 2(b) shows the PHBV nanofiber containing modified nanoparticles, indicating the production of more uniform and even composite nanofibers. It can be concluded that dispersion of bredigite nanoparticles were greatly improved within the nanofibers when the particles were treated with GPTMS.

The results of mechanical characterization of PHBV nanofibrous scaffold containing BR and T-BR nanoparticles including tensile strength, elongation at break and young modulus are summarized in Table 1. As can be seen from the results, incorporating BR nanoparticles in PHBV nanofibers resulted in lower mechanical properties compared to pure PHBV nanofiber. Generally, addition of ceramic nanoparticles at certain concentration can increase the mechanical performance but at higher concentration, particle aggregation and incompatibility may become the origin of the early failure, which decreases the mechanical performance of the composites nanofiber [1, 8, 13]. By incorporating modified nanoparticles into PHBV nanofibers, the tensile strength, young modulus and elongation at break of the composite nanofibrous scaffolds increased by 64%, 66% and 53%, respectively, compared to PHBV scaffolds reinforced with pure bredigite nanoparticles. When the nanoparticles disperse homogenously within polymer matrix, the matrix can transfer the concentrated stress to the nanoparticles effectively, consequently, improving the strength of the scaffolds.

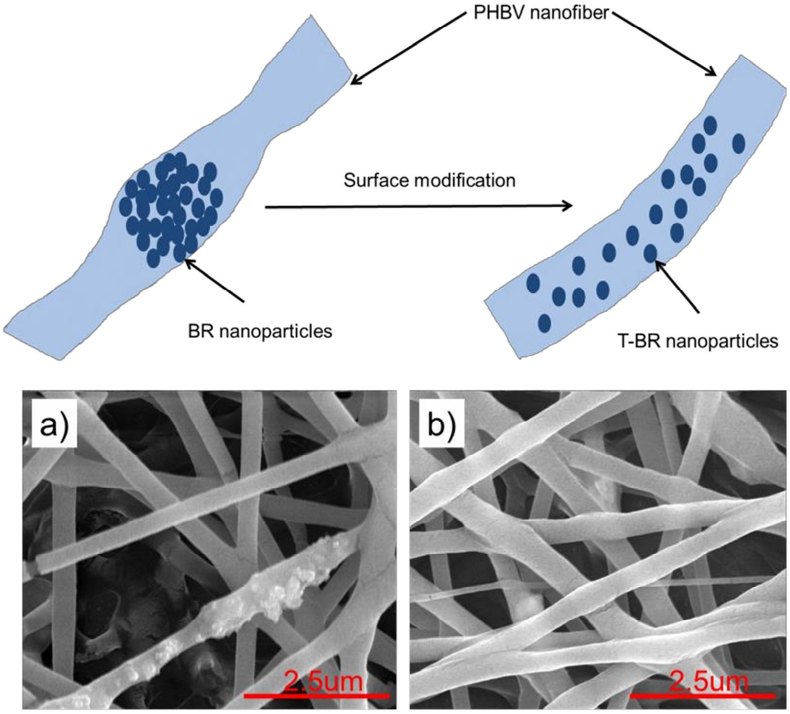

Fig. 2. Schematic illustration and SEM images of PHBV nanofibers containing 15% of a) BR and b) T-BR nanoparticles

Table 1. Mechanical properties of pure and composite PHBV nanofibrous scaffolds

	Elongation at break (%)	Young's Modulus (MPa)	Ultimate Strength (MPa)
PHBV	64.61±22.74	106.7±31.33	4.42±0.27
PHBV/BR	39.83±11.38	105.6±34.67	3.87±0.39
PHBV/T-BR	60.76±5.21	175.01±25.26	6.36±0.54

Dissolution behavior and bioactivity evaluation of biomaterials in SBF and examination of their apatite forming ability is beneficial prior applying them in vivo. Figure 3 illustrates the changes in Ca, P, Mg and Si ions available in the SBF solution containing composite nanofibrous scaffolds. The concentration of Mg and Si ions in the SBF increased due to dissolution of the bredigite nanoparticles. The dissolution of a bioactive material makes more nucleation sites on the surface of biomaterials for precipitation of apatite crystals, thus calcium phosphate deposits will form on the surface as a result of apatite formation which consume the P and Ca ions from the SBF solution [14, 15]. So it is expected that the concentration of Ca and P ions in SBF reduces during the soaking of bioactive materials. Such results were obtained in our study. The dissolution of a biomaterial is an important factor in formation of apatite deposits, the biomaterials with higher dissolution rate were shown to be more bioactive.

Furthermore, the materials dissolution causes more ion exchanges; consequently increase the pH of the SBF, which helps in formation of more apatite layer [14, 15].

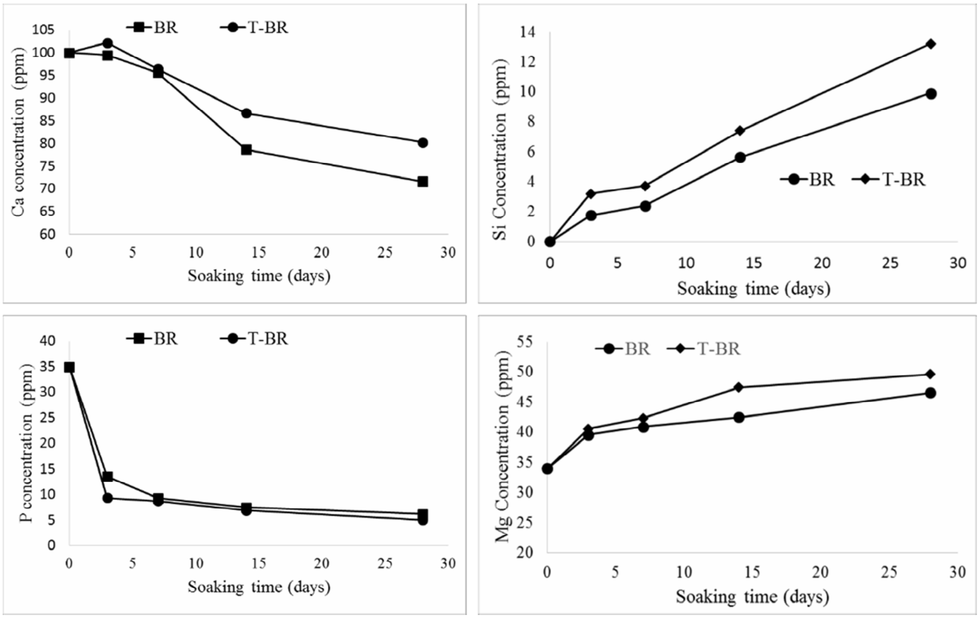

Fig. 3. The ion concentration changes in SBF containing PHBV composite nanofiber during soaking time

Degradation behavior of a scaffold is another important factor for application in bone tissue engineering. The degradation rate of a scaffold should match the rate of bone reconstruction [1, 9]. Figure 4 shows the SEM images of pure PHBV and PHBV nanofibers containing 15% BR and T-BR nanoparticles after 4 and 8 weeks soaking in PBS. As it can be seen PHBV nanofibers showed less morphological changes during degradation test, however, incorporation of either BR or T-BR nanoparticles increased the degradation of PHBV nanofibers. According to SEM images, PHBV composite nanofibers exhibited more changes in the fiber structure such as fiber breakage in some part and reduction in fiber diameter, proving the higher degradation of composite nanofibers compared to pure PHBV nanofibers. Bredigite is a slilicate based bioceramic whish is known to be able to hydrolyze in biological media, so presence of bredigite may increases the degradation rate of polymer matrix [8, 10].

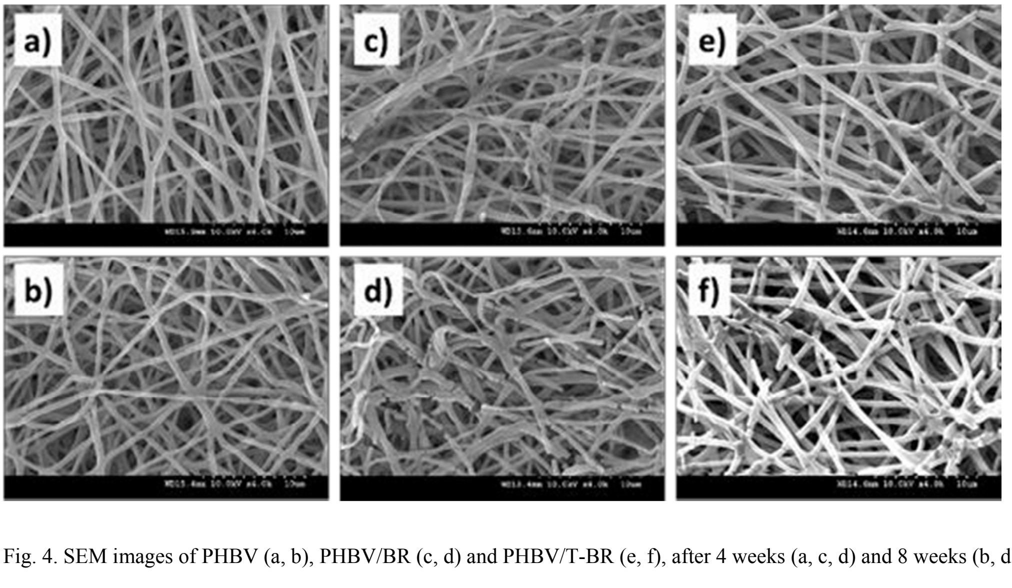

Fig. 4. SEM images of PHBV (a, b), PHBV/BR (c, d) and PHBV/T-BR (e, f), after 4 weeks (a, c, d) and 8 weeks (b, d, f) degradation in PBS

4. Conclusion

In this study bredigite nanoparticles were treated with a silane coupling agent to enhance its dispersibility and compatibility with polymer matrix. The successful modification of nanoparticles was proved by FTIR analysis. Electrospun PHBV nanofibers containing modified bredigite nanoparticles were successfully developed. According to SEM images, agglomeration of the nanoparticles within PHBV matrix reduced as a result of surface treatment. The results of mechanical test indicated the higher mechanical performance of PHBV scaffold containing treated nanoparticles compared to that field with pure bredigite. Enhanced bioactivity and degradation rate of PHBV nanofibrous scaffolds containing modified nanoparticles along with its improved mechanical properties make it a potential biomaterial for application in bone tissue engineering.

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