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The influences of polycaprolactone-grafted nanoparticles on the properties of polycaprolactone composites with enhanced osteoconductivity

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ABSTRACT

Bioceramic or inorganic nanoparticles made of SiO_2, TiO_2, SrO, and hydroxyapatite (HAP) have been reported to improve cell adhesion onto polymers. However, direct mixing of these nanoparticles with polymers often leads to their aggregation within the polymer matrix and subsequent deterioration of the material's mechanical strength. A novel method for modifying the surfaces of the nanoparticles by grafting ε-caprolactone using a ring-opening condensation reaction was developed to improve the interconnection of the nanoparticles within the polymer matrix. The mechanical studies showed that adding grafted nanoparticles into the polycaprolactone (PCL) matrix improved the initial mechanical strength. MTT assay and a live/dead stain showed higher cell viability in the tablets with grafted SiO_2, TiO_2, and HAP nanoparticles, except the SrO-containing tablets. The cell adhesion and alkaline phosphatase activity assay confirmed that the composite tablets with PCL-grafted HAP nanoparticles had better osteoconductivity. HE stains showed that composite tablets with PCL-grafted SiO_2, TiO_2, and HAP nanoparticles produce less immune response than the pure PCL. We thus conclude that a PCL matrix incorporating PCL-grafted HAP nanoparticles has enhanced mechanical strength, improved osteoconductivity, and a slower degradation rate than pure nanoparticles.

1. Introduction

For repairing bone defect, the bone substitute should have the capacity to promote migration of bone cells toward the scaffold [1,2]. Among the synthetic polymers investigated for use as scaffolds for generating tissue-engineered bony substitutes, the biodegradable polyesters poly (lactic-co-glycolic acid) (PLGA) and polycaprolactone (PCL) have been most widely used and investigated as bone substitutes [3–7]. However, implanted PLGA can induce inflammation and create a low-pH microenvironment, which has been attributed to the lactic and glycolic acid byproducts [8,9]. PCL is less acidic and thus more promising than PLGA as an implanted biomaterial. However, when used alone as a bone substitute, PCL has several disadvantages including insufficient strength and a lack of the desired bioactivity [10,11].

In order to overcome these disadvantages, increasing interest has currently been focused on polymer/ceramic composite materials as bone substitutes [12,13] such as electrospun PCEC/n-HA fibrous scaffold in tissue engineering application [14,15]. Some authors have advocated the introduction of hydroxyapatite (HAP), SiO_2, SrO and TiO_2 nanoparticles into the polymer matrix, which not only enhances the mechanical properties but also provides satisfactory osteoconductivity [16–20]. However, the compatibility and adhesion of SiO_2, TiO_2, SrO, and HAP with the polymers are rather poor [21]. Therefore, the search for an appropriate method to enhance the binding of nanoparticles to polymers has recently become an important aspect of research into bone substitutes. Several studies reported that polymers and particles incorporation could be enhanced by using grafted particles [22–24].

In this study, a new approach to modifying the surfaces of nanoparticles with PCL is introduced. PCL is a hydrophobic polymer, and the hydrophobic–hydrophobic interactions between the PCL on the grafted nanoparticles and the PCL matrix will make the composites more stable. This study focuses on grafted nanoparticles incorporated into a PCL matrix to form a bone substitute.

2. Materials and methods

2.1. Materials

PCL (Mw = 14,000) was purchased from Sigma. Hydroxyapatite (HAP), titanium oxide (TiO_2), strontium oxide (SrO), and silica

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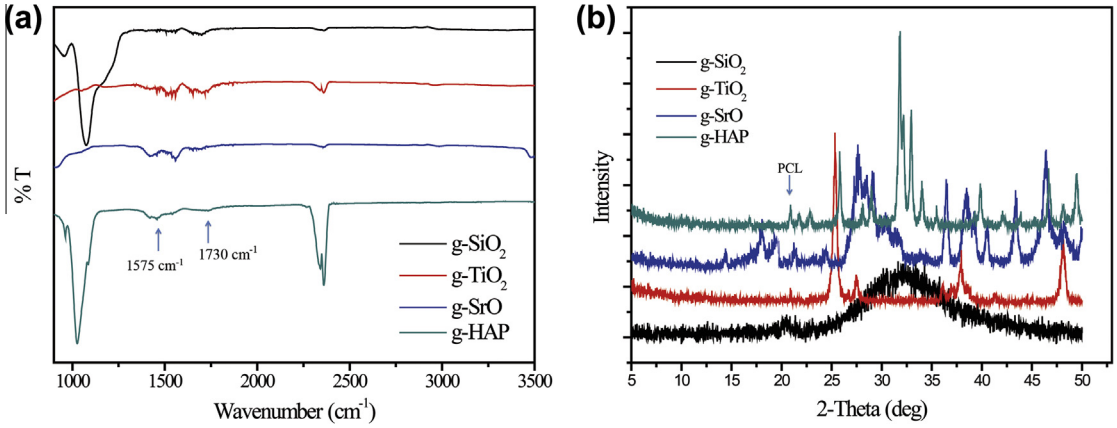

Fig. 1. (a) FTIR spectra of grafted nanoparticles. (b) The X-ray diffraction of grafted nanoparticles. The integral of the peak at 21.30° represents PCL.

(SiO_2) nanoparticles with average sizes of less than 200 nm were supplied by Aldrich Chemical. The ε-caprolactone monomer was obtained from Alfa Aesar. The live/dead cell double-staining kit was obtained from Fluka. Finally, 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was purchased from Merck, and all other reagents were of analytical grade.

2.2. Surface-grafting with ε-caprolactone monomer

The ε-caprolactone monomer was grafted onto the surfaces of the nanoparticles through a ring-opening condensation reaction in which stannous octoate ($Sn(Oct)_2$) was used as the catalyst. First, 2.0 g of nanoparticles (hydroxyapatite, titanium oxide, strontium oxide, or silica) was dispersed in 50 mL of dry toluene. Then, 5.0 g of the ε-caprolactone monomer was added into this solution, followed by 0.045 g of $Sn(Oct)_2$. Next, the mixture was stirred and heated to 130 °C under nitrogen. The reaction mixture was maintained at this temperature for 6 h, then cooled down to room temperature. The PCL-grafted nanoparticles (g-SiO_2, g-TiO_2, g-SrO, and g-HAP) were separated by centrifugation at 3500 rpm for 20 min. The precipitates were then washed with an excess of chloroform three times to completely remove the free PCL. Finally, the separated precipitate was dried in a vacuum oven overnight to remove the residual chloroform.

In order to characterize the PCL grafted on the surface of the nanoparticles, Fourier transform infrared spectrometry (FT-IR) was performed to determine whether there was an ester bond on the grafted nanoparticles. The grafting of PCL to each nanoparticle was assessed by performing X-ray diffraction (XRD, Ultima IV, Rigaku) measurements within $2\theta = 5–50°$ at a scan speed of 0.5°/min. The graft ratios of each nanoparticle were determined using a thermogravimetric analyzer (TGA, SDT Q600, TA Instrument) at a heating rate of 5 °C/min from room temperature to 700 °C under nitrogen gas flow.

2.3. Preparation of composite tablets

Different weight ratios of grafted nanoparticles (5 wt%, 10 wt%, and 20 wt%) were added to the PCL by feed ratio. The mixture was shaken by Vortex and flipped upside down five times before experiment. The total weight of each sample placed in the mold was 300 mg. The mixture in the mold was heated to 80 °C in a high-temperature furnace and maintained at this temperature for 30 min. After cooling to 60 °C, the iron was put above the mold to give a 100 N vertical force to reduce the bubble in tablet. Each tablet was 8 mm in diameter and 4 mm in height. A composite tablet with *x* wt% grafted nanoparticles is designated as particle-*x*.

2.4. Characterization of composite tablets

The tablets thermal properties, including their melting points (T_m) and enthalpy changes (ΔH), were measured by performing differential scanning calorimetry (DSC, Diamond DSC, Perkin Elmer) in unsealed pans at a heating rate of 5 °C/min. The degree of crystallinity (X_c) of the composite tablets was expressed in terms of the enthalpy of fusion (J/g) of the polymer sample determined from the DSC results and can be converted to a percentage using the following equation:

$$X_c = \frac{\Delta H_{\text{composite}}}{\Delta H_{\text{pure PCL}}} \times 100\% \quad (1)$$

The weight loss determines the degradation rate. First, the composite tablets were placed in separate release bottles. Then, 10 mL of phosphate-buffered saline (PBS, pH 7.4) was added to each bottle, and the bottles were placed in a shaking bath (37 °C, 100 rpm) for up to 12 weeks. Specifically, the composite tablets were retrieved at predetermined times (after 0, 1, 2, 4, 6, 8 and 12 weeks), dried under vacuum at room temperature, and weighed. The weight loss percent was calculated as shown in the following equation:

$$\text{weight loss} = \frac{W_d - W_o}{W_o} \times 100\% \quad (2)$$

where W_d is the residual weight at the predetermined time and W_0 is the original weight of the dried composite tablet. The acidity was assessed by measuring the accumulated pH of the supernatant at

Table 1
The thermodynamical properties of composites.

Composite	T_m[a] (°C)	ΔH[b] (J/g)	X[c] (DSC, %)
PCL-0	62.95	86.65	100.00
Si-05	64.28	82.67	95.40
Si-10	65.78	81.14	93.64
Si-20	65.94	69.01	79.64
Ti-05	63.95	84.26	97.24
Ti-10	65.46	64.30	74.21
Ti-20	65.93	55.43	54.04
Sr-05	64.78	83.45	96.31
Sr-10	65.94	75.31	86.91
Sr-20	66.44	69.81	80.57
HA-05	65.12	82.83	95.59
HA-10	65.27	71.74	82.79
HA-20	66.11	65.92	76.08

[a] Melting point.
[b] Enthalpy change.
[c] The degree of crystallinity.