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Synthesis and characterization of phthaloyl-chitosan-g-poly(L-lactide) using an organic catalyst

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Abstract

Biodegradable poly(L-lactide) (PLLA) was grafted through the hydroxyl groups of chitosan. Chitosan was converted to phthaloyl-chitosan (PHCS) by the phthaloylation of amino groups of chitosan in the first step, and then the graft copolymerization was performed using 4-dimethylaminopyridine (DMAP) as an organic catalyst. Both the effects of the molar ratios of DMAP catalyst and/or L-lactide monomer to PHCS, and the polymerization time on the graft copolymerization were investigated, respectively. The grafting content of PLLA within copolymer could be adjusted by the molar ratio of L-LA to PHCS in feed and the polymerization time, respectively, and the highest grafting content of PLLA was up to about 270%. Moreover, the PLLA grafts within copolymer existed in an amorphous structure, and they have better solubility in organic solvents than both chitosan and PHCS. Significantly, this will provide a convenient method to prepare new chitosan-based biohybrid without toxic metal contained.

Keywords: Chitosan; Chitosan-g-PLLA; DMAP catalyst; Graft copolymerization; Phthaloyl-chitosan-g-PLLA; Organic catalyst

1. Introduction

Chitosan is the fully or partially deacetylated polysaccharide of a naturally abundant chitin, known to be biocompatible, enzymatically biodegradable, nontoxic, and bioactive in animal tissues (such as anti-tumor and antibacterial functions). Recently, much attention has been paid to utilize chitosan and its derivatives in biomedical applications such as drug delivery vehicles, wound healing accelerator, surgical materials, and nerve regeneration agent (Kweon, Song, & Park, 2003; Wang, Ma, & Su, 2005; Yamaguchi, Itoh, Suzuki, Osaka, & Tanaka, 2003). Despite these promising potentials of chitosan, both poor mechanical and processing properties and its insolubility in common organic solvents have delayed its basic research and applications. Significantly, these drawbacks can be overcome through the adjustment of hydrophilicity–hydrophobicity balance of chitosan backbone, the chitosan-based graft copolymers, and chitosan blends with other polymers. For example, graft copolymerization of chitosan has been explored as a convenient method to develop novel hybrid materials, such as chitosan-graft-poly(ethylene glycol), chitosan-graft-poly(ε-caprolactone), chitosan-graft-polylactide, and so on (Bhattaraia, Ramaya, Gunna, Matsen, & Zhang, 2005; Liu, Wang, Shen, & Fang, 2005; Yao et al., 2003). An alternative approach is blending chitosan with biodegradable polylactones such as poly(ε-caprolactone) (Honma, Zhao, Asakawa, & Inoue, 2006).

Owing to its excellent mechanical strength, biocompatibility, and non-toxicity, biodegradable poly(L-lactide) (PLLA) has been widely used in drug delivery systems, surgical sultures, and tissue engineering (Albertsson & Varma, 2003; Dechy-Cabaret, Martin-Vaca, & Bourissou, 2004). However, the high crystallinity, the strong hydrophobicity of polymer backbone, especially *in vitro* and/or *in vivo* uncontrolled degradation rate of PLLA has greatly limited its applications as drug delivery carriers and scaffolds for tissue engineering (Cai, Zhao, Bei, & Wang, 2003). There-

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fore, it is promising to combine the hydrophilicity and the bioactive functions of chitosan with the good mechanical properties of PLLA to generate a new chitosan-PLLA biohybrid, which is expected to be applicable for a variety of biomedical purposes. Meanwhile, the hydrophobicity–hydrophilicity balance of chitosan-PLLA biohybrid can be easily adjusted by the grafting content of PLLA component within copolymer. For examples, Albertsson et al. reported the pH-sensitive hydrogels based on chitosan-g-poly(D,L-lactic acid) and/or poly(glycolic acid), which was synthesized by the graft copolymerization of chitosan with D,L-lactic acid and/or glycolic acid, while the degree of substitution of graft copolymers was very low (<20%) (Qu, Wirse'n, & Albertsson, 2000). Using the conjugation chemistry, Yao et al. synthesized chitosan-g-PLLA through aldehyde terminated PLLA and chitosan (Yao et al., 2003). However, both conjugation and reduction efficiency within this method were low, and it was difficult to purify the final product. Therefore, it is urgent to develop new methods to synthesize chitosan-g-PLLA copolymer with controlled grafting content of PLLA.

It is known that biodegradable PLLA can be generally synthesized from the ring-opening polymerization of lactides using stannous octoate ($SnOct_2$) as a catalyst (Dong, Qiu, Gu, & Feng, 2001; Wang & Dong, 2005). However, $SnOct_2$ catalyst can not be removed completely from the final products by a purification method involving dissolution and precipitation. This greatly limited its applications in biomedical field because organometallic tin compounds would induce some cell-toxicity in human body. Recently, different research groups explored new catalysts for the synthesis of polylactones and polylactides. For examples, Sanda et al. reported the synthesis of star-shaped poly(ε-caprolactone) using protonic acid as a catalyst (Sanda, Sanada, Shibasaki, & Endo, 2002). Liu et al. directly synthesized amino acid-terminated poly(ε-caprolactone) using amino acid as a catalyst (Liu & Liu, 2004). Interestingly, Hedrick et al. reported that nucleophilic organic compounds such as DMAP and phosphines could be used as a kind of highly efficient catalyst for the controlled synthesis of PLLA (Myers et al., 2002; Nederberg, Connor, Möller, Glauser, & Hedrick, 2001). In this paper, we intended to investigate DMAP as an organic catalyst to catalyze the graft copolymerization between phthaloyl-chitosan and L-LA monomer. Significantly, this will provide a convenient method to prepare chitosan-based biohybrid without toxic metal contained. The structure and physical properties of PHCS-g-PLLA have been preliminarily clarified by the techniques of FT-IR, ^{1}H NMR, and WAXD, respectively.

2. Experimental

2.1. Materials

Chitosan (viscosity = 200 CPs, degree of deacetylation (DD) = 80%) was purchased from Aldrich, and it was dried in vacuum at 100 °C for 8 h prior to use. L-Lactide (L-LA) was purchased from Aldrich and recrystallized from dry toluene before use. Both DMAP and Phthalic anhydride were purchased from GL Biochem Ltd (Shanghai, China) and used without further purification. Dimethyl sulphoxide (DMSO) was distilled under reduced pressure from calcium hydride and stored over molecular sieves.

2.2. Preparation of PHCS-g-PLLA

2.2.1. Preparation of PHCS

Chitosan (0.64 g) was heated with excess phthalic anhydride (1.66 g) in dried DMF (12 mL) to give PHCS according to the previously reported procedure (Kurita, Ikeda, Yoshida, Shimojoh, & Harata, 2002). It was obtained as a light-yellow powdery material, and the degree of substitution of phthaloyl groups within PHCS was determined to be about 1.1, calculated by ^{1}H NMR.

2.2.2. Graft copolymerization

PHCS macroinitiator was completely dissolved in DMSO (i.e. solving pre-treatment), and then both L-LA monomer and DMAP catalyst were quickly added into the solution. The tube was connected to a Schlenk line, where an exhausting–refilling process was repeated three times. The tube was put into an oil bath at 120 °C with vigorous stirring for a certain time, then cooled to room temperature. The resulting mixture was extracted with toluene for 4 h to remove the PLLA homopolymer, and then the purified graft copolymer was dried in vacuum at 100 °C for 8 h. The grafting content of PLLA (the grafting content of PLLA = $(W_{\text{PHCS-g-PLLA}} - W_{\text{PHCS}})/W_{\text{PHCS}}$) was determined gravimetrically and by ^{1}H NMR, respectively. A typical example follows: PHCS (0.100 g, 0.367 mmol GlcN units) was completely dissolved in DMSO (1 mL), and then both L-LA (1.058 g, 7.347 mmol) and DMAP (0.180 g, 1.475 mmol) were added into the solution. The tube was connected to a Schlenk line, where an exhausting–refilling process was repeated three times. The tube was put into an oil bath at 120 °C with vigorous stirring for 32 h, and then cooled to room temperature. The resulting graft copolymer was extracted with toluene for 4 h to remove the PLLA homopolymer, and then dried in vacuum at 100 °C for 8 h (0.371 g, the grafting content of PLLA within copolymer = 271%).

2.3. Characterization of PHCS-g-PLLA

FT-IR spectrum was recorded on Fourier-transform infrared spectrometer. PHCS and its graft copolymer were mixed with KBr and pressed to a plate for measurement. ^{1}H NMR spectroscopy was performed on a Varian Mercury-400 spectrometer. Tetramethylsilane was used as an internal standard. Both PHCS and its graft copolymers were dissolved in d_6-DMSO solvent. Wide angle X-ray diffraction (WAXD) patterns of powder samples were obtained at room temperature on a Shimadzu XRD-6000

X-ray diffractometer with a CuKα radiation source (wavelength = 1.54 Å). The supplied voltage and current were set to 40 kV and 20 mA, respectively. Samples were exposed at a scan rate of $2\Theta = 4^\circ\ min^{-1}$ between $2\Theta = 5^\circ$ and 40°.

3. Results and discussion

3.1. Synthesis of PHCS-g-PLLA

It is known that chitosan can be easily converted to phthaloyl-chitosan (PHCS) by the phthaloylation of amino groups of chitosan (Kurita et al., 2002). Introduction of phthaloyl groups could reduce the inter- and/or intramolecular hydrogen bonds, which resulted in the solubility of chitosan in organic solvents such as DMSO and DMF. Thus, this will facilitate the hydroxyl groups of chitosan to initiate the graft copolymerization of L-lactide (L-LA) monomer under homogeneous conditions. The degree of substitution of phthaloyl group within PHCS was determined to be about 1.1 from ^{1}H NMR analysis. This suggests that all the amino groups at C-2 within chitosan completely reacted with phthalic anhydride (Fig. 1a).

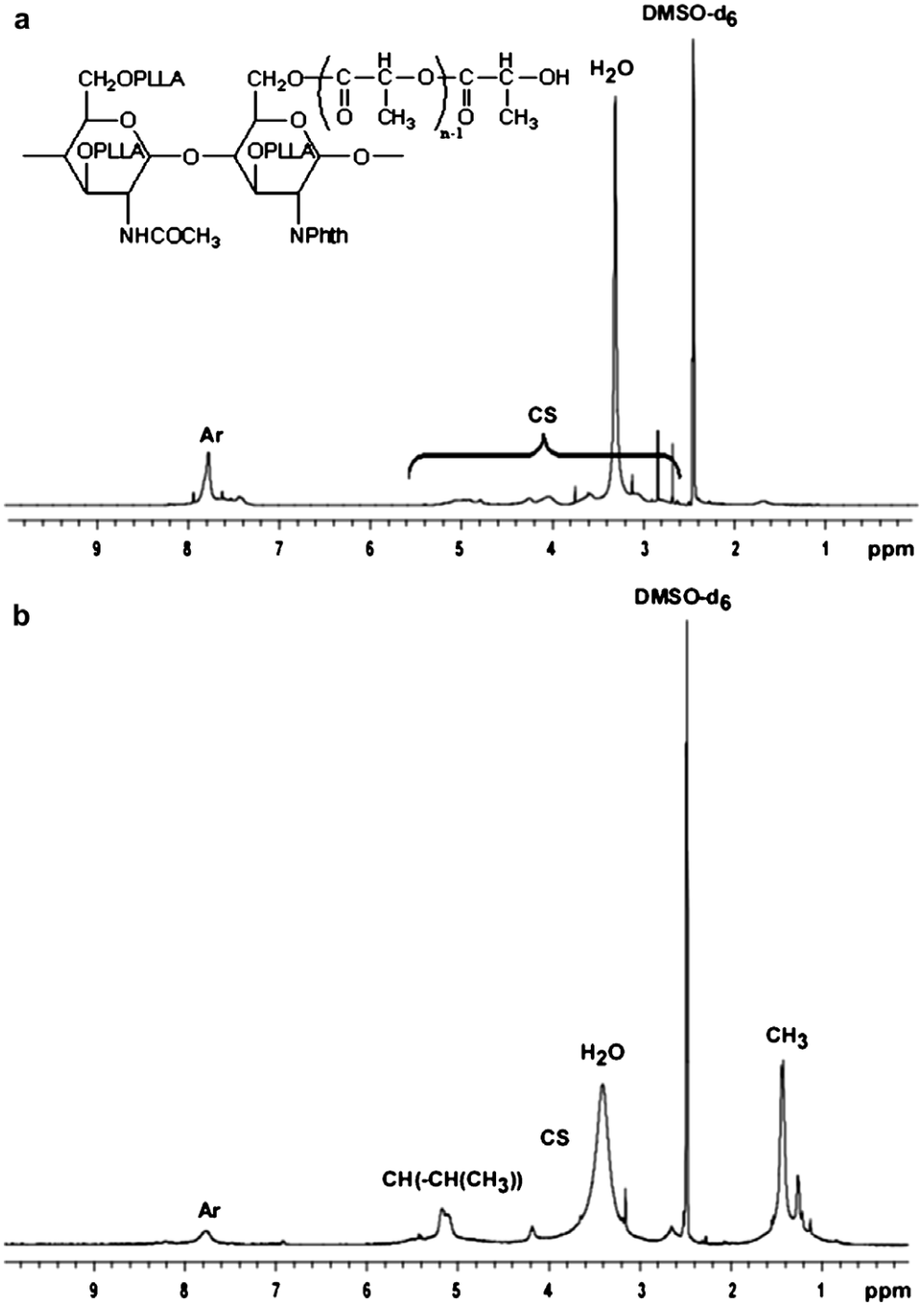
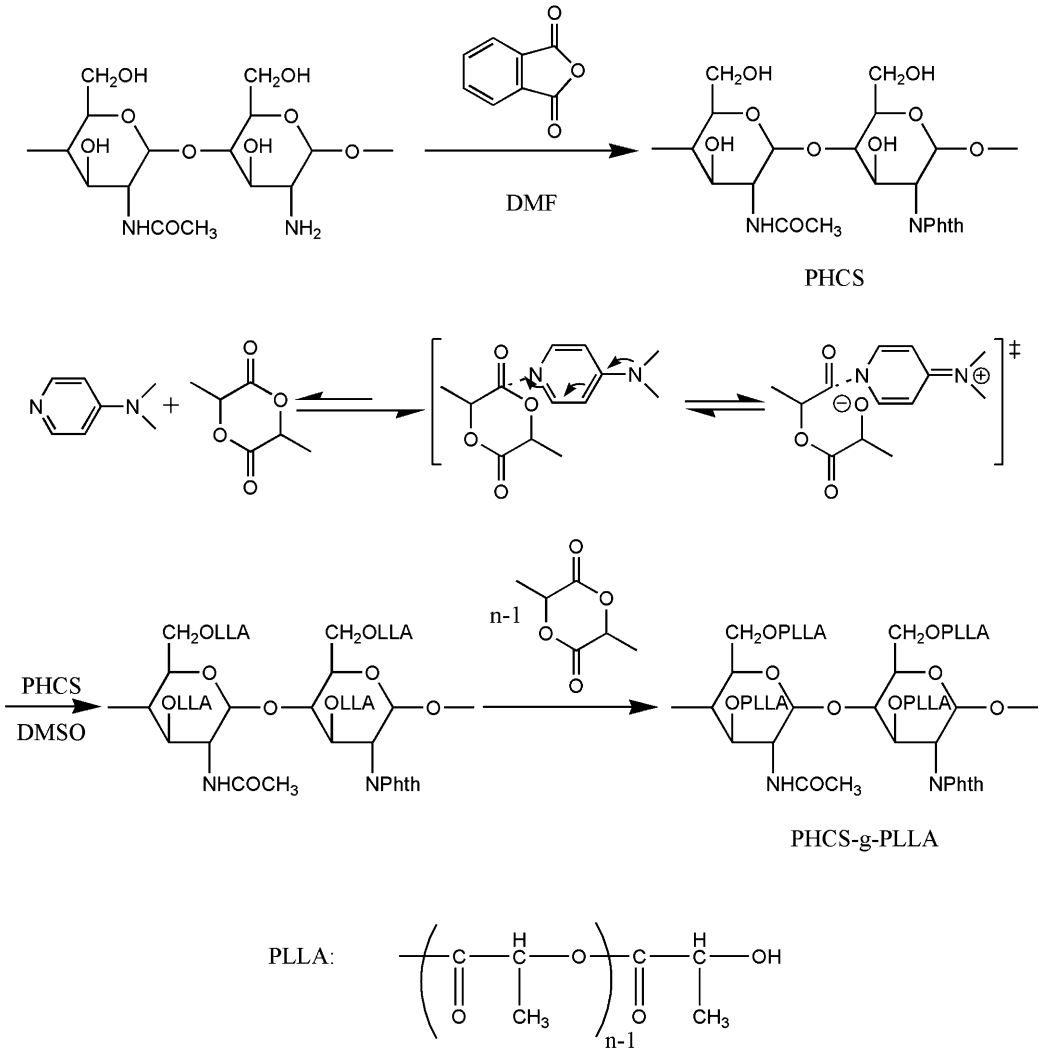


Fig. 1. The ^{1}H NMR spectra of (a) PHCS and (b) the PHCS-g-PLLA copolymer (sample 11, Table 1).

PHCS-g-PLLA copolymer was prepared by the graft copolymerization of L-LA with PHCS using DMAP as a catalyst in DMSO at 120 °C, as shown in Scheme 1. The results of the graft copolymerizations were summarized in Table 1. The effect of the molar ratio of DMAP to PHCS (according to the pyranose units of PHCS) on the graft copolymerization was first investigated ([DMAP]/[PHCS] = 2, 4, 8, and 16, respectively). Obviously, the grafting content of PLLA within PHCS-g-PLLA copolymer was highest when the molar ratio of DMAP catalyst to PHCS macroinitiator was 4. No graft copolymer could be obtained with the increase of DMAP used (such as [DMAP]/[PHCS] = 8 and 16). This was probably attributed to the superfluous DMAP used, which would induce the precipitation of PHCS from the DMSO solution. In addition, the solving pre-treatment also has an apparent effect on the graft copolymerization, where PHCS was completely dissolved in DMSO before both L-LA monomer and DMAP catalyst were added. For example, sample 8 with solving pre-treatment obviously had higher grafting content of PLLA than sample 5 without pre-treatment (i.e. PHCS, L-LA, and DMAP were just mixed together). This was attributed to that PHCS macroinitiator was completely dissolved in DMSO, thus the graft copolymerization occurred under homogeneous solution. This also indicated that the solving pre-treatment was an important factor for the efficient graft copolymerization. Therfore, all the other copolymerizations were carried out under homogeneous solution, where the molar ratio of DMAP to PHCS was 4.

The effect of the polymerization time on the graft copolymerization was also studied, as shown in Fig. 2. When the molar ratio of PHCS to L-LA was 1:20, it was demonstrated that the grafting content of PLLA within copolymer was highest when the polymerization time was 32 h. Meanwhile, both the results of samples 6 and 6′ showed that the experiments were repeated under the polymerization conditions used. If the polymerization time increased to 68 h, the grafting content of PLLA within copolymer inversely decreased. This was attributed to the occurrence of transesterification reaction of PLLA grafts within copolymer, which resulted in the production of some



Scheme 1. A tentative plausible polymerization mechanism for the preparation of chitosan-g-PLLA using DMAP as a catalyst.

Table 1
Synthesis of PHCS-g-PLLA using DMAP as a catalyst at 120 °C

Sample	[PHCS]/[L-LA] (mol/mol)	[PHCS]/ [DMAP] (mol/mol)	[PHCS]/[DMSO] (g/ml)	[L-LA]/[DMSO] (g/ml)	Time (h)	Grafting content[c] (wt.%)	Grafting content[d] (wt.%)	Yield of PLLA[e] (wt.%)
1[a]	1:40	1:2	0.05	1.056	48	72	–	–
2[a]	1:40	1:4	0.05	1.056	48	115	–	–
3[a]	1:40	1:8	0.05	1.056	48	–	–	–
4[a]	1:40	1:16	0.05	1.056	48	–	–	–
5[a]	1:20	1:4	0.1	1.056	48	115	–	–
6[b]	1:20	1:4	0.1	1.056	24	145	–	–
6′[b]	1:20	1:4	0.1	1.056	24	142	–	25.6
7[b]	1:20	1:4	0.1	1.056	32	271	264	31.3
8[b]	1:20	1:4	0.1	1.056	48	252	–	36.6
9[b]	1:20	1:4	0.1	1.056	68	110	–	46.2
10[b]	1:5	1:4	0.1	0.264	24	86.5	–	–
11[b]	1:10	1:4	0.1	0.528	24	117	109.4	–
12[b]	1:40	1:4	0.1	2.112	48	132	–	–

[a] No solving pre-treatment was done before both monomer and catalyst was added.
[b] PHCS was first dissolved in DMSO (i.e. solving pre-treatment) before both monomer and catalyst was added.
[c] The grafting content of PLLA = $(W_{\text{PHCS-g-PLLA}} - W_{\text{PHCS}})/W_{\text{PHCS}}$, which was determined gravimetrically.
[d] The grafting content of PLLA was determined by ^{1}H NMR.
[e] The PLLA homopolymer was produced within the graft copolymerization of L-LA with PHCS, and the yield of PLLA = $(W_{\text{PLLA}})/W_{\text{L-LA}} \times 100\%$.

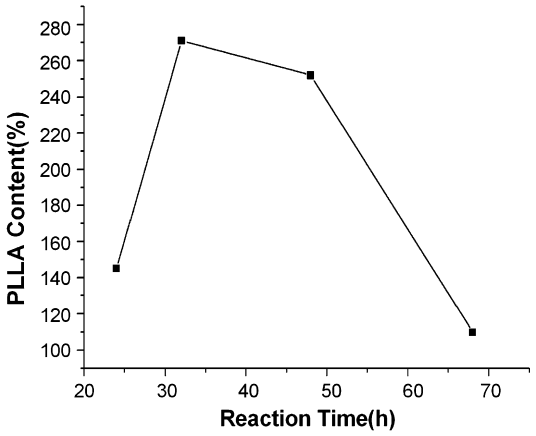


Fig. 2. The effect of the polymerization time on the graft copolymerization.

PLLA homopolymer (Albertsson & Varma, 2003). This phenomenon can be to some extent explained by the fact that the resulting PLLA homopolymer within the graft copolymerization increased with the increase of the polymerization time (Table 1).

The trials with different molar ratios of L-LA monomer to PHCS macroinitiator were further investigated (samples 6, 10, and 11). It could be concluded that the grafting content of PLLA within copolymer increased with the increasing molar ratio of L-LA to PHCS in feed. While sample 12 to some extent showed a decreased tendency even with longer polymerization time. This was probably attributed to that both PHCS and L-LA could not mix very well in DMSO when higher L-LA concentration was used (such as [L-LA]/[DMSO] = 2.112 g/mL, Table 1). In all, PHCS-g-PLLA copolymers with different grafting content of PLLA can be synthesized via the graft copolymerization between PHCS and L-LA using DMAP as a catalyst in 120 °C. Consequently, the polymerization pathway was postulated to occur through a monomer-activated mechanism (Nederberg et al., 2001). Initiation occurs when the hydroxy group of chitosan reacts with the lactide–DMAP complex to form the monoadduct, and polymerization proceeds when the terminal ω-hydroxyl group continues the propagation (Scheme 1).

3.2. FT-IR analysis of PHCS-g-PLLA

As shown in Fig. 3, the PHCS precursor showed the characteristic peaks at 1712 and 1776 cm^{-1} of the phthalimido groups and at 721 cm^{-1} of the aromatic ring. Compared to the FT-IR spectrum of PHCS, the obtained PHCS-g-PLLA presented new absorption peaks around at 1197 and 1256, 1750, and 3000 cm^{-1}, which were assigned to the symmetric C–O–C stretching modes of the ester group, the carbonyl group, and C–H (–CH(CH$_3$)) of the PLLA grafts, respectively. This implied the successful graft copolymerization of L-LA through the backbone of PHCS. Meanwhile, the relative intensity of hydroxyl

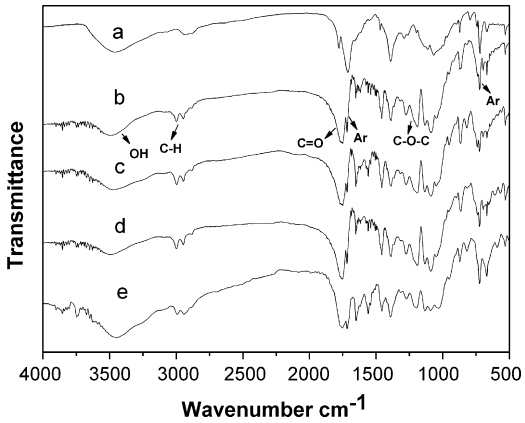


Fig. 3. The FT-IR spectra of PHCS and the PHCS-g-PLLA copolymers: (a) PHCS; (b) sample 5; (c) sample 8; (d) sample 7; (e) sample 10.

groups within PHCS at 3450 cm^{-1} grew less with the increasing grafting content of PLLA while that of carbonyl groups at 1750 cm^{-1} increased with it.

3.3. ^1H NMR analysis of PHCS-g-PLLA

The ^1H NMR spectra of PHCS and PHCS-g-PLLA copolymer were shown in Fig. 1b. Compared with PHCS, the ^1H NMR spectrum of the graft copolymer showed new proton signals at 5.15 ppm and 1.40 ppm assigned to C–H (–CH(CH_3)) and CH_3 of PLLA grafts, respectively.

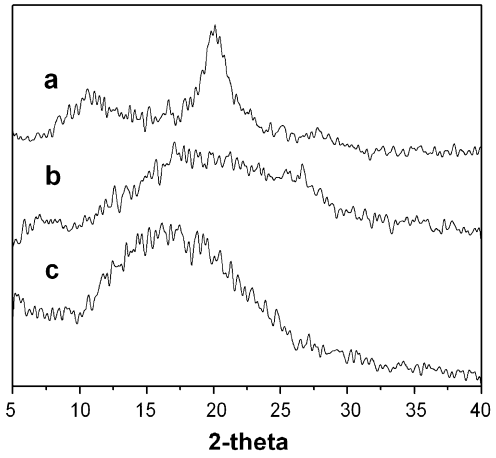


Fig. 4. Wide angle X-ray diffraction patterns of chitosan (a), PHCS (b) and the PHCS-g-PLLA copolymer (c, sample 7).

This further confirmed the successful grafting of PLLA through the backbone of PHCS. Moreover, the grafting content of PLLA within copolymer was determined by the relative signal intensities of PLLA units and PHCS units. The grafting content of PLLA within copolymer could be calculated as follows:

$$\text{PLLA grafting content } \% = \frac{(I_{1.25\,\text{ppm}} + I_{1.4\,\text{ppm}}) \times \text{MW}_{\text{l-LA}}}{3 \times 2 \times I_{2.8\,\text{ppm}} \times \text{MW}_{\text{PHCS}}} \times 100$$

3.4. Physical properties of PHCS-g-PLLA

As illustrated in Fig. 4, chitosan had two reflection peaks at $2\theta = 10°$ and 20°, no obvious peak was detected for PHCS. This indicated that the introduction of phthaloyl groups destroyed the crystalline patterns of chitosan. For the PHCS-g-PLLA copolymer (sample 7), there existed a broader peak around at $2\theta = 12.5$–22.5°, which should be the reflection of PLLA grafts. Notably, no strong signals were observed at around $2\theta = 16.5°$ and 19°, which were indicative of the presence of PLLA crystals (Cai, Wang, & Dong, 2006). This suggested that PLLA within PHCS-g-PLLA copolymer was grafted through the backbone of chitosan in a well-proportioned way, and it existed in an amorphous structure.

3.5. Solubility of PHCS-g-PLLA

In comparison with the original chitosan and PHCS, the PHCS-g-PLLA copolymers exhibited an improved solubility in some organic solvents, even in acetone and chloroform (Table 2). This also suggests that the PLLA grafts not only improved the hydrophobic property of PHCS, but also possibly reduced the inter- and/or intra-molecular hydrogen bonds of chitosan backbone. This will provide the PHCS-g-PLLA biohybrid to be easily fabricated into micro- and/or nano-particles, which are expected for the biomedical applications.

4. Conclusion

PHCS-g-PLLA biohybrid was prepared via ring-opening graft copolymerization between L-LA monomer and PHCS using DMAP as a catalyst in DMSO at 120 °C. The grafting content of PLLA within copolymer could be

Table 2
Solubility of chitosan, PHCS, and PHCS-g-PLLA

Sample	CH_3OH	Ethanol	$CHCl_3$	DMSO	DMF	THF	Toluene	Acetone	CH_2Cl_2
Chitosan	−	−	−	−	−	−	−	−	−
PHCS	−	−	−	+	+	−	−	−	−
PHCS-g-PLLA (sample 6)	±	−	+	+	+	±	−	+	+
PHCS-g-PLLA (sample 8)	±	−	+	+	+	±	−	+	+

+, soluble; ±, milky-like dispersion; −, insoluble.

adjusted by the feed ratio of L-LA to PHCS and the polymerization time, respectively, and the highest grafting content of PLLA was up to about 270%. The PLLA grafts within copolymer existed in an amorphous structure, and they have better solubility in organic solvents than both chitosan and PHCS. Significantly, this will provide a convenient method to prepare new chitosan-based biohybrid without toxic metal contained, which should be easily fabricated into micro- and/or nano-particles for the biomedical applications.

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