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Porous poly-L-lactic acid scaffold reinforced by chitin fibers

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Summary

In this study, we reinforced the porous poly-L-lactic acid (PLLA) scaffold by chitin fibers. To enhance the strength of the scaffold further, we launched the treatment linking PLLA and chitin fibers by Dicyclohexylcarbodimide (DCC). The structures of the composites with and without link treatment were characterized by Scanning Electron Microscopy (SEM) and porosity. The chemical characteristics of the chitin fibers with and without link treatment were evaluated by Fourier-transformed infrared (FTIR) spectroscopy and X-ray photoelectron spectroscopy (XPS). The mechanical strength was measured by compressive tests. The results suggest that after linked with PLLA successfully, the chitin fibers can reinforce the scaffold much more effectively. The linked scaffold, with the compressive strength 4 times than PLLA, also has better structure and pore size than the scaffold without link treatment. So it is a kind of very potential appropriate scaffold for tissue engineering.

Introduction

Tissue engineering requires cellular components, scaffolds, and growth factors. Scaffolds act as a substrate for cellular attachment, proliferation and differentiation. Growth and differentiation factors guide the appropriate development of the cellular components. Various synthetic alternatives such as metals, ceramics and polymers have been tried as scaffolds for many years [1, 2]. The scaffold lies at the heart of all the new tissue engineering approaches [3, 4].

Poly-L-lactic acid (PLLA) is a kind of nontoxic, biodegradable material which is used as scaffold material widely in the tissue engineering. However, as scaffold material, PLLA has several obvious weaknesses: biodegrading too fast, acidic degradation product, and hydrophobic. The chitin material, which is alkalescent, can neutralize the acidity caused by PLLA degradation, which will reduce the hydrolization speed and eliminate the inflammations, and to prolong the exciting of the material in the body. Chitin has been widely applied in biomedical applications, such as wound dressings and drug delivery systems [5, 6] on account of its nontoxic and biocompatible nature. One of the most interesting effects of chitin on wound healing is the formation of granulation tissue with angiogenesis [7]. It is reported that chitin induces fibroblasts to release interleukin-8, which is involved in migration and proliferation of fibroblasts

and vascular endothelial cells [8, 9]. So scaffolds composed of PLLA and chitin may create an appropriate environment for the regeneration of tissue.
Regarding the scaffold, it is generally agreed that a highly porous microstructure with interconnected pores and a large surface area is conducive to tissues in growth [10]. For bone regeneration, pore sizes between 100 and 350μm and porosities of more than 80% are preferred [11, 12]. But a good scaffold should possess not only satisfactory porosity but also appropriate mechanical properties. Only if it possesses good mechanical properties, can the scaffold keep its shape and characters after being embedded in the body [13-15].
In this paper, we reinforced the porous PLLA scaffold by high-strength chitin fibers. To strengthen the scaffold further, we launched the treatment linking PLLA and chitin fibers by oil-soluble Dicyclohexylcarbodimide (DCC). The results suggest that after linked with PLLA successfully, the chitin fibers can reinforce the scaffold much more effectively.

Experimental

Materials

Chitin fibers (60% deacetylated, diameter 12.5 μm, tensile-strength 550MPa) were purchased from Donghua University, China. PLLA (M_W: 10^5) was purchased from Shandong medical appliance factory. Dicyclohexylcarbodimide (DCC), dioxane and other chemicals were purchased from Beijing Chemical Co.Lt d., China, which has a nation medicament permit.

Preparation of the PLLA /chitin fibers scaffold without linking

PLLA was dissolved in dioxane (pore-forming agent) at the concentration of 0.08g/ml. Then, the chitin fibers, not linked with PLLA, were added to PLLA solution gradually while the liquid was stirred by magnetic force. And then, the liquid was dispersed ultrasonically for 45min. Finally, the liquid was lyophilized for 12h.

Preparation and characterization of the PLLA /chitin fibers scaffold with linking

The chitin fibers, PLLA and DCC (1:4:2 M/M/M) were dissolved into dichloromethane. After the mixture was stirred by magnetic force at 0℃ for 2h, the linked PLLA/chitin fibers was taken out and washed by dichloromethane for more than thrice. Then the linked substance was air-dried.
PLLA was dissolved in dioxane (pore-forming agent) at the concentration of 0.08g/ml. Then, the linked PLLA/chitin fibers substance was added to PLLA solution gradually while the liquid was stirred by magnetic force. And then, the liquid was dispersed ultrasonically for 45min. Finally, the liquid was lyophilized for 12h.
Characterization of the fibers linked with PLLA was performed using the methods such as Fouriter-transformed infrared (FTIR) spectroscopy, X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM) and mechanical strength measurement. XPS analysis was performed using a Perkin-Elmer 5600 electron spectroscope for chemical analysis (ESCA). FTIR spectra were recorded with a Bio-Red FIS135 FTIR spectrometer. The scaffolds were observed by JSM-6460LV SEM under the voltage of 20KV. The compressive strength was measured on electronic universal material testing machine. The loading rate was 0.5mm/min. The size of the dry samples was Φ8.5×15mm.

Measurement of the porosity

The porosity was measured by liquid substitution method. The liquid used in this study was isopropanol. The porosity was calculated by the form:

$$\varepsilon = (V_1 - V_3)/(V_2 - V_1) \quad (1)$$

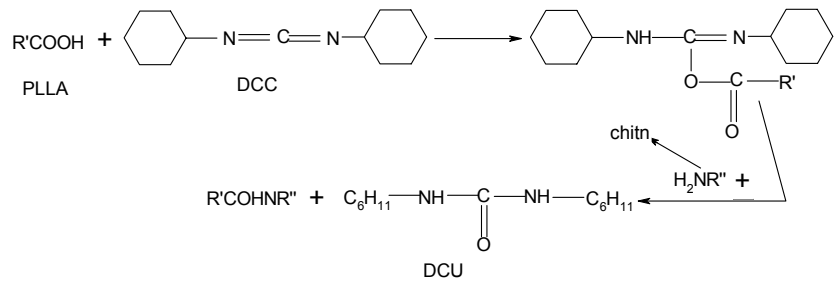
Note: ε, the porosity of the scaffold; V_1, the volume of isopropanol before the scaffold was put in; V_2, the volume of the liquid after the scaffold was put in; V_3, the volume of isopropanol after the liquid was pressed into the pore of the sample and the sample was taken out of the liquid.

Results and discussion

Study of the materials by FTIR spectra

The FTIR spectra obtained from pure chitin fibers, the chitin fibers mixed mechanically with PLLA and the chitin fibers linked with PLLA by DCC are given in Fig.1. In the spectrum of pure chitin fibers, six absorption bands at the frequencies of 3324, 3082, 1585, 1380, 1152 and 1040 cm^{-1} can be observed. Generally, I bands (1040 cm^{-1}) originate from skeletal vibrations involving the C–O stretching. The II bands (1152 cm^{-1}) arise from anti-symmetric stretching of the C–O–C bridge. The III bands (1380 cm^{-1}) originate from $–CH_2$ bending. The IV bands (1585 cm^{-1}) arise from $–NH_2$ bending. The other two amines, arising from the stretching vibrations of N–H group, of a medium to weak intensity, appear at 3324 and 3082 cm^{-1}, respectively. After mixed mechanically with PLLA (Fig.1b), the values of frequencies of these bands hardly change. But The new bands with weak intensity, arising from C=O stretching, appears at 1770 cm^{-1}, the reason of which might be that a little PLLA was bonded with the fibers by their molecular force. So the fibers can reinforce the material to a little extent, only after mixed mechanically with PLLA.

After linked with PLLA by DCC (Fig.1c), the values of frequencies of these bands for the pure fibers also hardly change, which indicates the link doesn't change the intrinsic characteristics of the chitin fibers. But two important amines, originating from –CONHR bending, appear at 3275 and 1680 cm^{-1}, respectively, the reason of which is that R′COHNR″ generated after the chitin fibers were linked with PLLA by DCC, showed in Scheme 1. This result suggests that the chitin fibers were successfully linked with PLLA by DCC. Besides this, the bands (1770 cm^{-1}) arising from C=O stretching, increase obviously comparing with that in fig. 1b. This result indicates that more PLLA was bonded with the fibers by the link.



Scheme 1. The link course of chitin fibers and PLLA by DCC

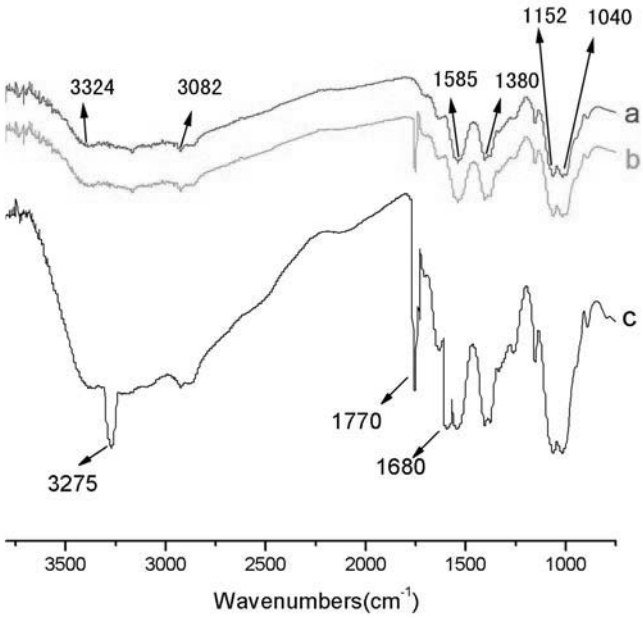


Figure 1. FTIR spectra of: (a) pure chitin fibers; (b) the chitin fibers mixed mechanically with PLLA and (c) the chitin fibers linked with PLLA by DCC

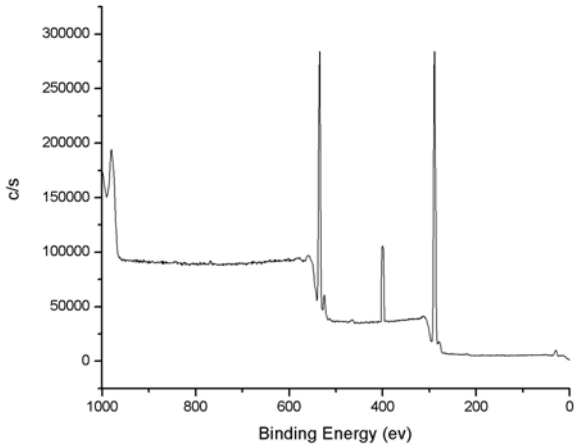
ESCA survey scan spectra

The changes in chemical structure of the matrices were further investigated by ESCA spectra (Fig.2). The control chitin fibers surface shows carbon (binding energy: 287.8 eV), oxygen (binding energy: 534.8 eV) and nitrogen (binding energy: 400 eV) peaks, as expected (Fig.2a). The PLLA surface shows carbon (binding energy: 287.8 eV), oxygen (binding energy: 534.8 eV), as expected (Fig.2b). For the linked the fibers and PLLA surface, an increased carbon peak and decreased nitrogen and oxygen peaks, comparing with those of the pure chitin fibers, were observed (Fig.2c).
The chemical compositions of the controls and the chitin fibers linked with PLLA calculated from the ESCA survey scan spectra, were shown in Table 1. The carbon content (74.19%) of the chitin pure fibers was decreased (70.16%) because of the introduction of PLLA in the matrix and because of the low C/O ratio in the PLLA, whereas the nitrogen content (3.50%) decreased (2.85%) in the fibers linked by PLLA because of zero nitrogen content in the PLLA.
These results suggest that the chitin fibers were successfully linked with PLLA.

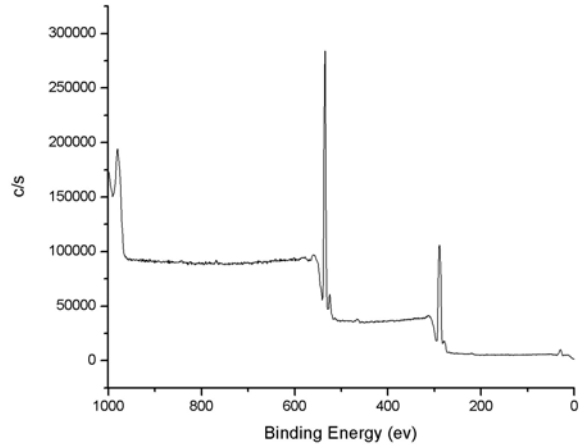
Table 1. Elemental composition of the samples

Sample	Composition (%)			
	C	N	O	S
The pure chitin fibers	74.19	3.50	22.15	0.16
PLLA	63.69	0	36.09	0.22
The chitin fibers linked with PLLA	70.16	2.85	26.82	0.17

(a)



(b)



(c)

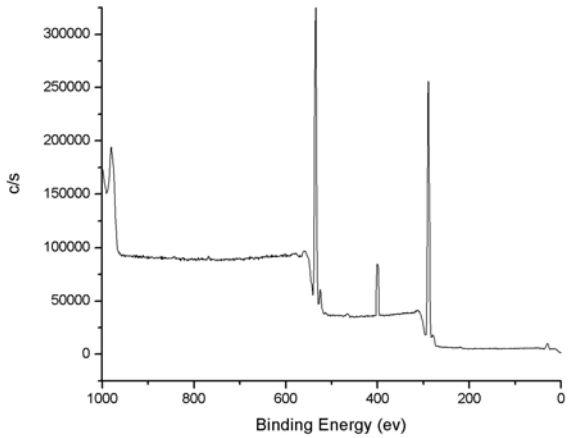
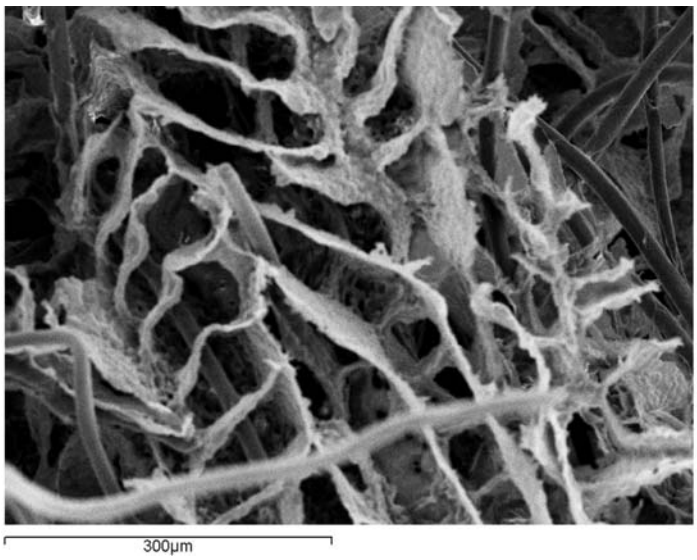


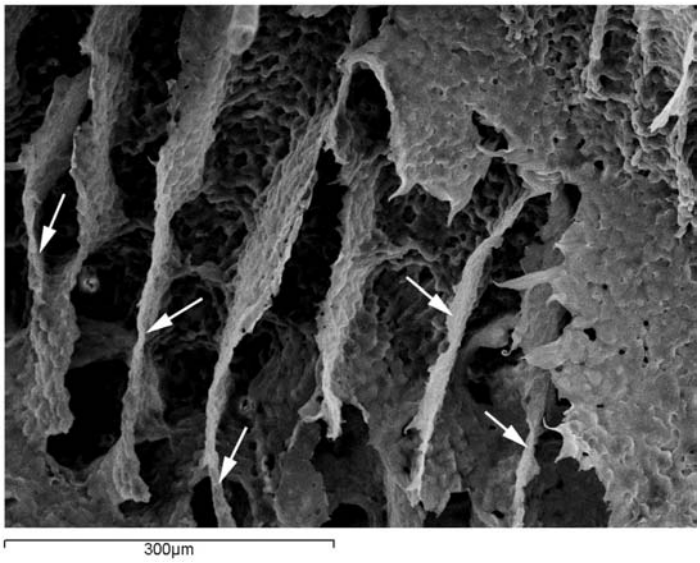
Figure 2. ESCA spectra of: (a) the pure chitin fibers; (b) PLLA and (c) the chitin fibers linked with PLLA by DCC

Scanning electron micrograph of the reinforced matrices

Scanning electron micrographs can sometimes display the conjoint condition of all the components in the samples [16, 17]. Therefore, in this study, scanning electron micrographs of the samples with and without treatment were shown in Fig.3. The chitin fibers in samples without link treatment (Fig.3a) can be identified easily and don't bond with PLLA very well. But the chitin fibers in samples with link treatment



(a)



(b)

Figure 3. Scanning electron micrograph of: (a) the samples without link treatment; (b) the samples with link treatment

(Fig.3b) can not be identified easily (shown in the figure by the arrows) and have been integrative with PLLA. So after linked with each other, the chitin and PLLA integrate much better than they do only after mixed mechanically. A good conjoint condition of all the components in complex materials reinforced by some materials is very helpful for satisfactory mechanically properties [18, 19]. So the link treatment is very beneficial to reinforcement of PLLA by the chitin fibers.

Furthermore, it can be seen from Fig.3 that after the link treatment, pore and structure of the samples are more even than they do only after mixed mechanically because the link treatment makes the chitin fibers and PLLA integrative. Uniformity of pore and structure of the scaffold will benefit growth of tissues and degradation of the scaffold. From Fig.3, it can also be seen that after the link treatment, pore size (between 100 and 200μm) of the samples is bigger than it does only after mixed mechanically and has already obtained the request of pore size for a perfect scaffold, which is conducive to tissues in growth.

Compressive strength of the matrices

The compressive strength of the matrix in tissue engineering applications is of great importance due to the necessity of structural stability to withstand stress incurred during culturing *in vitro* and implanting *in vivo*. It can also significantly affect the specific biological functions of cells within the engineered tissue [20-22].

Therefore, the compressive strengths of the two kinds of samples were shown in Fig.4. In one kind of samples, the fibers and PLLA were mixed mechanically and in the other samples, the fibers and PLLA were linked by DCC. Generally speaking, the mechanical strengths of the two kinds of samples both increase, with the increase of volume content of the fibers, respectively. But the strength of the samples with link treatment increases more rapidly than that of the samples, in which the fibers and PLLA were only mixed mechanically. Though the strength of the samples with link treatment begin to decrease when the volume content of the fibers is more than 40 percent because much more fibers have leaded to uneven component and structure of

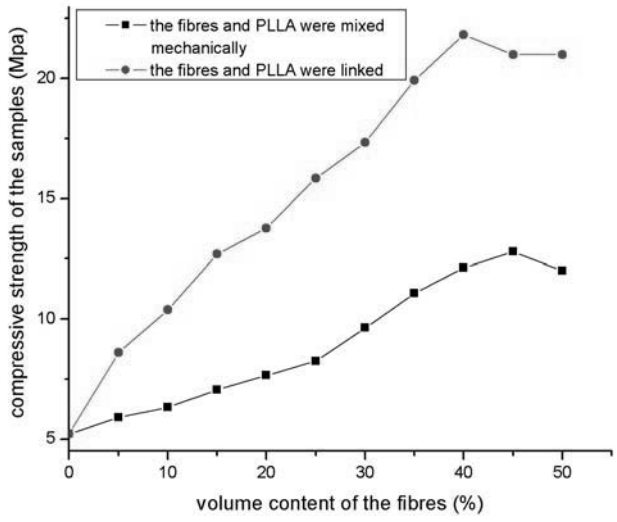


Figure 4. The compressive strengths of the two kinds of samples

the samples, the fibers can still reinforce the PLLA scaffold more than 4 times while the fibers, only after mixed mechanically with PLLA, can merely reinforce the PLLA scaffold about 2.5 times. So after linked with PLLA, the chitin fibers can reinforce the scaffold much more effectively.

Porosity of the reinforced matrices

For the scaffolds of tissue engineering, porosity is one of very important parameters. Satisfactory porosity of more than 80 percent is a distinct symbol of a perfect scaffold [23-25]. Therefore, porosity of samples with and without link treatment was investigated respectively, the results of which was shown in table 2. The results indicate that the porosity of the two kinds of samples both decreases while the volume content of the fibers increases. But the porosity of two kinds of samples is both more than 80 percent, even when the volume content of the fibers is 50 percent. Though the porosity of the samples with link treatment is a little lower than that of the samples without link treatment, it has already obtained the request of porosity for a perfect scaffold.

Table 2. Porosity of the samples

Volume content of the fibers (%)	Porosity of the reinforced scaffold (%)	
	Samples with link treatment	Samples without link treatment (only mixed mechanically)
10	91.0±0.2	91.0±0.2
20	90.2±0.1	90.5±0.4
30	86.5±0.3	89.2±0.1
40	84.2±0.2	88.5±0.3
50	82.3±0.1	82.5±0.2

Conclusions

Porous poly-L-lactic acid (PLLA) scaffold can be reinforced by chitin fibers. PLLA can be linked with chitin fibres by Dicyclohexylcarbodimide (DCC). After linked by DCC successfully, the scaffold of PLLA and chitin fibers has not only much higher mechanical strength but also better structure and pore size than it does after only mixed mechanically. So the linked scaffold, with the compressive strength 4 times than PLLA, might be a kind of very potential appropriate scaffold for tissue engineering.

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