Electrospun Non-Woven Fabrics of Poly(ε-caprolactone) and Their Biodegradation by Pure Cultures of Soil Filamentous Fungi

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Summary: The biodegradation of electrospun nano-fibers of poly(ε-caprolactone) was firstly investigated, using pure-cultured soil filamentous fungi. In the biochemical oxygen demand test, the biodegradation of the nano-fiber exceeded 20–30% carbon dioxide generation. The biodegradabilities decrease with increase of the mean fiber diameter.

Keywords: biodegradation; electrospinning; nano-fibers; poly(ε-caprolactone); soil fungi

Introduction

Electrospinning is a unique technique able to prepare the polymer fibers having diameters in the ranges from nano- to a few micrometers.[1] Non-woven fabrics composed of electrospun nano-fibers have a large surface area per unit mass and a small pore size.[2,3] Biodegradable non-woven fabrics have been recently focused because of their wide ranges of applications.

Poly(ε-caprolactone) (PCL) is one of the most important class of biodegradable polymer which exhibits a high-level biodegradability in almost all given situations, considering *in vitro*, *in vivo*, and environmental usage.[4,5] As for the electropsun non-woven fabric of PCL, its biodegradation property has been less known. We report here the preparation of the electrospun non-woven fabrics of PCL and their biodegradabilities with respects to environmental usage.

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The purposes of this study are as follows; (i) The first evaluation of the environmental biodegradability of electrospun PCL nano-fibers; (ii) Laboratory experiments of PCL biodegradation using the single strained soil filamentous fungi; (iii) Relationship between the structural characteristics and the biodegradabilities of PCL nano-fibers.

Experimental

Materials

PCL (M_n 80,000) was purchased from Aldrich (Milwaukee, USA). This material was dissolved in a mixture of methylene chloride and *N,N*-dimethylformamide (85/15, vol.-%) at room temperature. The viscosity of PCL solution was determined by using the rheometer (DV III, Brookfield Co., USA) equipped with a spindle No. 3 at 100 rpm at 20°C.

Electrospinning of Poly(ε-caprolactone)

PCL solution prepared was poured in a 5-mL syringe attached to a capillary tip of about 1 mm diameter. The copper wire connected to an anode was inserted into the solution and a cathode was attached to a grounded rotating metallic collector. The electric field was produced by a high-voltage power supply (CPS-60 K02v1, Chungpa EMT, Co., Republic of Korea).

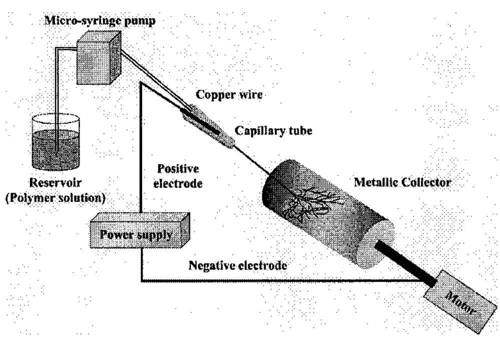


Figure 1. Electrospinning apparatus.

PCL non-woven fabrics were made from solutions with viscosities of 150, 210, and 310 centipoise under identical conditions with 15 kV (applied voltage) and 120 mm (distance between capillary tip and collector). After electrospinning, non-woven fabrics produced were dried in a vacuum oven for 1 week to remove the residual solvent.

Microscopic Observation of Fungal Growth

Seven species of single strained soil filamentous fungi, *Aspergillus oryzae*, *Penicillium caseicolum*, *P. citrinum*, *Mucor* sp., *Rhizopus* sp., *Curvularia* sp., and *Cladosporium* sp. were employed, according to our previous article.[6] The non-woven PCL sheet samples were cut into pieces (10 × 10 mm^2). Three pieces were placed in a dish and then the modified Czapeck medium (1/100-reduced carbon source) and the sporangia suspensions were then added at 25°C. The growth of the microorganisms and the collapse of the nano-fiber accompanying the biodegradation were observed using a scanning electron microscope.

Quantification of Biodegradability

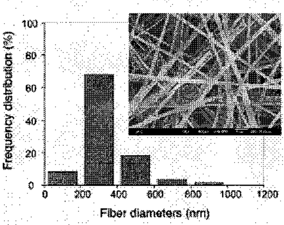
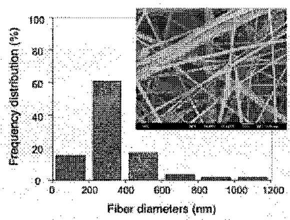
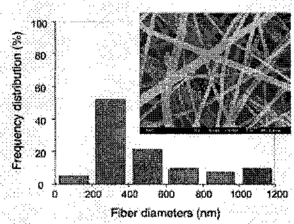
The biochemical oxygen demand (BOD)-biodegradation test was conducted using the method reported by Doi et al.[7] The non-woven PCL samples (7–8 mg) were placed in a BOD reactor, and the modified Czapeck medium and sporangia suspensions of the fungi were then added.
The biodegradation test was carried out for 30 days, and the BOD was continuously measured as the CO_2 evolution volume. The BOD-biodegradation of the fiber sample was calculated using an equation: Degradation (%) = $100 \times (BOD^t - BOD^b) / TOD$, where BOD^t and BOD^b are values of the fiber sample and blank test, respectively. The TOD is the theoretical oxygen demand.

Results and Discussion

Characterization of Non-Woven Fabrics

Table 1 represented the distribution of fiber diameters in the electrospun non-woven fabrics of PCL. Three kinds of the non-woven fabrics having nano-scale fibers were prepared from the PCL solutions at three different viscosities, 150, 210, and 310 cPs. At the solution viscosity of 150 cPs, the peak fraction in the diameter distribution located around 200–400 nm, and the frequency distribution was approximately 70 %. The mean fiber diameter in the electrospinning at 150 cPs was 330 nm. SEM photographs visually proved that the thicker fibers were prepared in the higher viscosity. The estimated mean fiber diameters obtained in the 210 and 310 cPs conditions were 360 and 510 nm, respectively. The sample names were designated as the PCL330, PCL360, and PCL510, from their mean diameters.

Table 1. Characterization of electrospun PCL nan-woven fabrics

Solution viscosity	150 cPs	210 cPs	310 cPs
			
Mean diameter	330 nm	360 nm	510 nm

Fungal Growth on PCL Nano-Fibers

The PCL non-woven fabrics were subjected to microbial degradation in a culture with seven species of soil fungi. Among the fungi tested, the *Mucor* sp. and *Rhizopus* sp. remarkably degraded the PCL non-woven fabrics, and the process of the degradation could be observed under a scanning electron microscope. Figure 2 shows the SEM images during the growth of *Mucor* sp. on the PCL360 sample. Before biodegradation (Figure 2a) , the non-woven fabrics exhibited the fibril structure with nano-scale diameter. In the initial phase, the filament of *Mucor* sp. became being growing on the surface of the PCL nano-fibers. After a 7-days cultivation, progress in surface coverage by the fungal body is clearly observed. The secretes from the fungal body covered the surface and digested the nano-fibers.

Further cultivation after 14 days confirmed a complete coverage of the nano-fiber surface by the fungal filaments. The maturation of the *Mucor* sp. was finally represented by the development of fungal spores (Figure 3b). These results indicated that the life cycle of *Mucor* sp. could be run during the cultivation on the PCL nano-fiber as well as that the electrospun PCL non-woven fabrics could be the nutrition for the *Mucor* sp. The metabolism of the PCL digests in the fungal body was subsequently examined by the BOD tests.

Table 2. Biodegradabilities of PCL non-woven fabrics by soil filamentous fungi

	CO_2 production[a)] (micro mol)			Biodegradation (%)		
Filamentous fungi	PCL510	PCL360	PCL330	PCL510	PCL360	PCL330
Rhizopus sp.	64	75	116	17	20	31
Mucor sp.	45	53	75	12	14	20
Cladosporium sp.	42	46	60	11	12	16
Aspergillus oryzae	14	14	25	4	4	7
Curvularia sp.	14	10	14	4	3	4
Penicillum caseicolum	12	9	12	3	2	3
Penicillium citrinum	4	4	6	1	1	2

a) data after a 28-days cultivation.

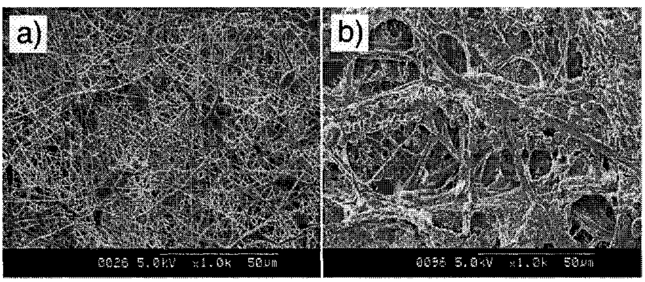


Figure 2. SEM observation of biodegradation process of PCL non-woven fabrics by *Mucor* sp.: (a), before degradation; (b), after incubation for 14 days.

Effect of PCL Nano-Fiber Diameters on Biodegradation by Soil Fungi

In the BOD tests, four species of fungi, *Rhizopus* sp, *Mucor* sp, *A. oryzae*, and *Cladosporium* sp. degraded all three kinds of PCL samples, producing the CO_2 gas. To summarize the results from the BOD tests, the relationship between the mean diameter of the PCL nano-fiber and the biodegradability was examined (Table2). In the all four fungi concerned, the degree of biodegradation is significantly increased when the mean fiber diameter below 360 nm. The biodegradability of the PCL non-woven fabrics will be constant above the mean diameter of 510 nm. This result suggests that the biodegradability of the non-woven fabrics of PCL might be regulated by the fiber diameter lower than 360 nm.

Conclusion

In the present study, we firstly performed the laboratory experiments on the biodegradability of electrospun non-woven fabrics of PCL using seven species of soil filamentous fungi. The following findings were obtained; (i) Single strained soil fungi were grown on the PCL non-woven fabrics, degrading the PCL nano-fiber and utilizing them as the nutrition, and (ii) The biodegradability of the PCL non-woven fabrics depends on their nano-fiber diameters. This finding could be expand to general electrospinnig system dealing with polyester-based materials, including the polymers of L-lactate and *β*-hydoxybutylate, which are oriented to the environmental uses.

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[1] J. Doshi, D. H. Reneker, *J. Electrostat.* **1995**, *35*, 151.
[2] K. H. Lee, H. Y. Kim, Y. M. La, D. R. Lee, N. H. Sung, *J. Polym. Sci.* **2002**, *40*, 2259.
[3] K. H. Lee, H. Y. Kim, Y. J. Ryu, K. W. Kim, S. W. Choi, *J. Polym. Sci.* **2003**, *41*, 1256.
[4] D. Goldberg, *J. Environ. Polym. Degrad.* **1995**, *3*, 61.
[5] A. C. Albertsson, R. Renstad, B. Erlandsson, C. Eldsater, S. Karlsson, *J. Appl. Polym. Sci.* **1998**, 70, 61.
[6] K. Ohkawa, M. Yamada, A. Nishida, N. Nishi, H. Yamamoto, *J. Polym. Environ.* **2000**, *8*, 59.
[7] Y. Doi, K. Kasuya, H. Abe, N. Koyama, S. Ishiwatari, K. Takagi, Y. Yoshida, *Polym. Degrad. Stab.* **1996**, *51*, 281.