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Preparation and Characterization of Nanofibrous Membranes of Poly(D,L-lactic acid)/Chitin Blend for Guided Tissue Regenerative Barrier

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Abstract: Nanofibrous membranes of poly(D,L-lactic acid)/chitin blend were prepared by electrospinning for a barrier of guided tissue regeneration. A miscible solution was obtained by the blending chitin-salt complex into 1-methyl-2-pyrrolidone solution of poly(D,L-lactic acid). The properties of the blend were examined for nanofibrous fabrication. The viscosity of the blend solution was increased significantly due to chain entanglement despite the low ratio of chitin to poly(D,L-lactic acid). An interaction between two polymeric compositions was confirmed by Fourier transform infrared spectroscopy. X-ray diffraction detected an appreciably ordered microstructure in the nanofiber of the blend. A membrane of thinner nanofibers was fabricated by electrospinning the chitin blend. The permeability of the membranes was examined using bioactive model compounds.

Keywords: poly(D,L-lactic acid), chitin, blend, nanofiber, membrane, GTR barrier.

Introduction

Guided tissue regeneration (GTR) is a well-established dental therapy that promotes periodontal augmentation for filling up an insufficient volume of periodontal bone in a lesion, which promotes reconstruction of the neobone using a barrier membrane to guard the lesion from invasion of fibrous connective tissues.[1-3] As the barrier membrane prevents the down-growth of fibroblasts or epithelial cells that have fast proliferative capacity, spaces are allocated for the in-growth of cells derived from the residual periodontal ligament and bone marrow, with subsequent extracellular matrix deposition and bone mineralization.[4]

Conventional materials used as the GTR barrier are non-degradable expanded polytetrafloroethylene (ePTFE), biodegradable collagen, and degradable aliphatic polyesters including poly(lactic acid) (PLA). For more than a decade, thin ePTFE membrane has been used as a barrier which can be safely applied clinically as a biologically inert material. Despite this advantage, however, non-degradable membrane must be removed by a secondary operation attended with a dehiscence.[5,6]

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While bioresorbable collagen membrane has excellent cell affinity and biocompatibility, it has an antigenic problem and a risk of carrying bovine spongiform encephalopathy transmission.[7,8] Although bioresorbable aliphatic polyester-based polymers have poor biocompatibility in comparison with collagen, they are non-antigenic and have sufficient mechanical properties.[9,10] These synthetic bioresorbable polymers such as poly(D,L-lactic acid) (PDLLA) are advantageous in that their properties can be designed to accommodate gradual degradation and metabolism.[11,12]

Chitin, poly(β-(1-4)-N-acetyl-D-glucosamine), is a natural polysaccharide. It can be degraded by chitinase and lysozyme, and has exceptionally low immunogenicity and excellent biological properties.[13,14] Histological findings suggest that chitin has good cell adhesion and stimulates the migration of polymorphonuclear and mononuclear cells and accelerates cytokine production of fibroblasts.[15]

On the other hand, the electrospinning method has recently attracted a great deal of attention to produce non-woven membranes of nanofibers. A non-woven-type matrix composed of nanofibers is architecturally similar to the extracellular matrix in which collagen multifibrils of a nanofiber scale (50-500 nm) are composed of a three-dimensional network structure together with proteoglycans. The electro-

spinning technology is well suited to process synthetic polymers for biomedical applications.[16-19]

Polymer blending is an attractive alternative for producing miscible polymeric composites with newly tailored properties. It is expected that the composite of PLA blended with chitin has optimal properties that complement each other for the functionality and the fabrication of GTR membranes. To obtain materials comprising of the two components simultaneously, various methods were examined; synthesis of chitin-graft-PLA copolymer,[20] blending PLA with alkanoyl chitosan,[21,22] mixing chitin into melt PLA,[23] and separate electrospinning of chitin solution[24] or poly(vinyl alcohol) chitin blend solution[25] with poly(lactide-*co*-glycolide) solution on one target. However a homogeneous blend of both polymers has not yet been reported despite of both hydrophobic properties. The primary reason for this may lie in the low solubility of rigid chitin chains arising from its strong intermolecular hydrogen bonding and ladder structure. From the same reason, successful electrospinning of chitin also has not yet been reported in open literature despites its potential applications in the medical area.

In this content, we present a novel method of preparing a miscible blend solution of PDLLA and chitin, thereby fabricating nanofibrous membranes by electrospinning of the blend solution. We also investigated the suitability of the properties of the PDLLA/chitin blend for nanofibrous fabrication.

Experimental

Materials. PDLLA copolymer was supplied by Cargill Dow Polymer LLC. The content of L-lactide and D-lactide in PDLLA were 81.9% and 8.1%, respectively. The measured viscosity average molecular weight of PDLLA was about 200,000. Chitin (Mw 400,000) was purchased from Fluka. The chemical structures of the polymers are shown in Figure 1. Chitin was complexed with lithium chloride before dissolving. 1-Methyl-2-pyrrolidone (NMP) was used as co-solvent of PDLLA and chitin complex. Fluorescein isothiocyanate bovine serum albumin (FITC-BSA: Mw 66,000) and fluorescein isothiocyanate dextran (FITC-Dextran: Mw 4,000) were used as model protein and model polysaccharide for permeability of nanofiber membranes. The simulated body fluid (SBF) was prepared by dissolving reagent-grade NaCl, $NaHCO_3$, KCl, K_2HPO_4, $MgCl_2$, $CaCl_2$, and Na_2SO_4 in deionized water, and by buffering at pH 7.4 with tris(hydroxymethyl)aminomethane and 1 M hydrochloric acid at 36.5 °C as reported in the our previous paper.[26]

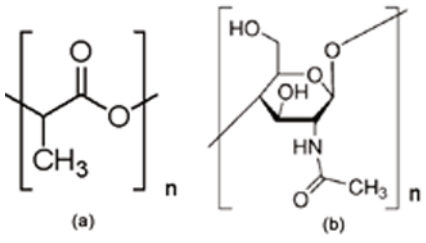

Figure 1. Chemical structures of (a) poly(D,L-lactic acid) and (b) chitin.

Blend Solution. PDLLA was dissolved in NMP which has relatively low cytotoxicity and local tissue irritation. Chitin formed a coordinate covalence with lithium chloride in NMP solution. The chitin-salt solution was prepared from dissolving 1.2% chitin in NMP in which 5% LiCl was dissolved. The blend solution of 15% total polymer concentration was prepared from fairly mixing the two polymeric solutions. The blend ratio of each solution and the chitin concentration of the blend solution are shown in Table I. The homogeneous solutions were used as spinning dope for nanofiber fabrication.

Electrospinning. Nanofibrous membranes were prepared by a typical electrospinning apparatus with a dope-injecting syringe and drum target. The feeding rate of the blend solution was fixed to 0.12 mL/h. A voltage of 2 kV/cm was applied to the dope. Variously fabricated nanofibrous web membranes were collected for 3 h on the grounded metal drum target rotating at 35 rpm. The fabricated membranes were washed several times by methanol and deionized water. The specimen dried thoroughly under vacuumed conditions thereafter.

Analyses. Chemical analysis of the blend was carried out by a fourier transform infrared spectrophotometer (FTIR; Bruker IFS 66/Bruker, KBSI) within a range of 4000 to 400 cm^{-1} at 0.3 cm^{-1} resolution. The specimens for FTIR were prepared by the KBr powder method. Crystallographic studies were carried out with a wide-angle X-ray diffractometer (WAXRD; Rigaku Dmax 2000V) using monochromatic CuK_α radiation at 40 kV and 30 mA. The fibrous morphologies were examined by scanning electron microscopy (SEM; Hitachi S-4200) after pre-coating the specimen with platinum in a Hitachi E1010 ion sputter. Viscosities of the blend solutions were analyzed from the relations of shear stress or apparent viscosity to strain rate, which were measured by rotary cone viscometer for high viscosity (Tokimec, Visconic EHD). The permeability of bioactive model compounds was performed in SBF using a diffusion apparatus that was composed of a donor cell, membrane stage, and receptor cell.[27] FITC-BSA and FITC-Dextran were used as

Table I. The Blend Ratio between 1.2% Chitin Solution and PDLLA Solution, and Chitin Concentration of the Blend Solution

Blend Ratio of 1.2% Chitin/PDLLA Solution (%)	0/100	2/98	4/96	8/92	12/88	15/85	20/80
Concentration of Chitin (μmol)	0	0.6	1.2	2.4	3.6	4.5	6.0

the model protein and model polysaccharide, respectively. The permeating process was performed in an incubator at 37 °C with stirring. The permeating concentration was measured the absorbency of fluorescein isothiocyanate excitation at 495 nm.

Results and Discussion

Composition. As shown in the FTIR spectra (Figure 2), the absorption bands at 1042, 1092, 1129, 1187, 1270, 1382, 1458, 1743, 2947, and 2997 cm^{-1} are the characteristic vibration bands of δC-O, ν_sC-O-C, $\gamma_{as}CH_3$, ν_{as}C-O, δCH, $\delta_s CH_3$, $\delta_{as}CH_3$, νC=O, $\nu_s CH_3$, and $\nu_{as}CH_3$ vibration of PDLLA.[28] The absorption bands at 1027~1071, 1310, 1375, 1552, 1656 cm^{-1} are the characteristic bands of γCH_3, ν amide III, νC-O, ν amide II, and ν amide I of chitin.[14] For PDLLA/chitin blend, most bands of C-O and CH_3 groups appeared at same wavenumber with those of PDLLA, while the stretching vibration band of C=O shifted to a higher wavenumber of 1750 cm^{-1} by +7 cm^{-1}. Also, the bands of amide I and II stretching vibration of chitin appeared at 1649 cm^{-1} and 1559 cm^{-1}, respectively, which also shifted by ca. ±7 cm^{-1}. These vibrational changes, of which PDLLA carbonyl and chitin amide II are increased and chitin amide I is decreased, might be attributed to an interference effect between neighbor PDLLA carbonyl groups and chitin amide amino groups in blend.

Microstructure. Crystalline microstructure of biomaterial greatly influences the degradation of the implant in the body. For confirmation of the crystalline microstructure of PDLLA/chitin blended nanofiber, wide-angle X-ray diffractograms were analyzed by powder reflection method. The results are shown in Figure 3. The microstructure of PDLLA nanofiber is the amorphous phase, which had very broad scattering at 2θ=10°~30° due to a random copolymer of D-lactide and L-lactide. On the other hand, chitin has the semi-crystalline phase, which showed typical diffractions at 2θ=9°, 12°, 19° corresponding to (020), (021), (110) reflections.[29] In the diffractogram of nanofiber of the PDLLA/chitin blend, a newly broad diffraction was observed appreciably, which had a peak 2θ=17.5° extending 10°~30°. This indicated that there was not a perfect crystalline phase, but the blending of chitin chains into amorphous PDLLA phase triggered an ordered microstructure in electrospinning process of the nanofiber.

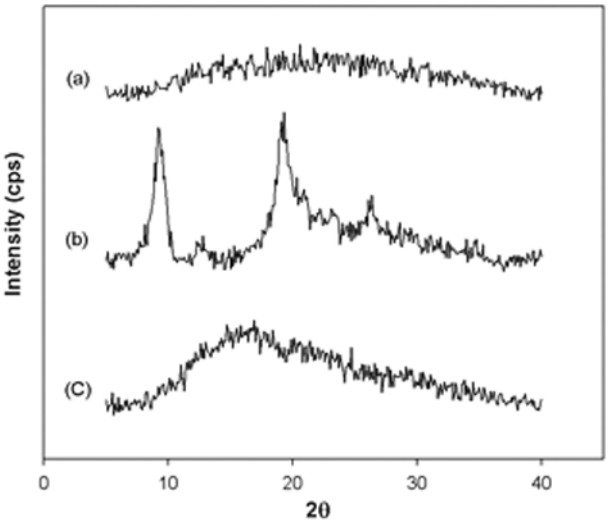

Figure 3. WAXRD diffractograms of nanofiber membranes for (a) PDLLA, (b) chitin, and (c) PDLLA/chitin blend.

Viscosity. The viscosity behaviors of blend solutions were investigated according to chitin content. The viscosity of spinning solution (namely dope) is one of main factors that affect fibrous fabrication in all fibrous spinning systems because a normal fibrous fabrication can be obtained within a definite range of viscosity of dope. Figure 4 showed shear stress curves to strain rate of blend solutions. The shear

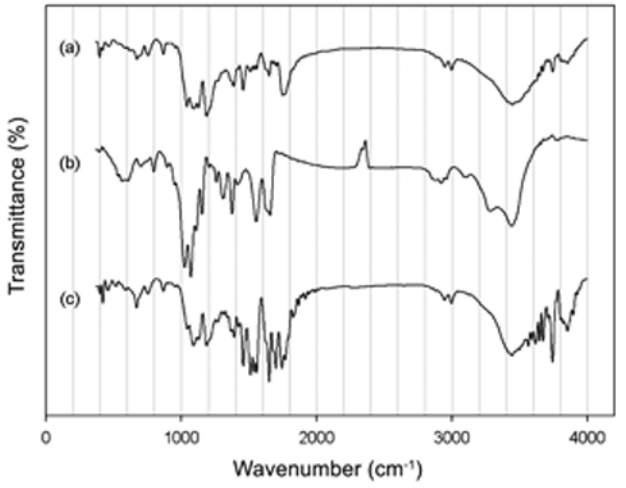

Figure 2. FTIR spectra of (a) PDLLA, (b) chitin, and (c) PDLLA/chitin blend.

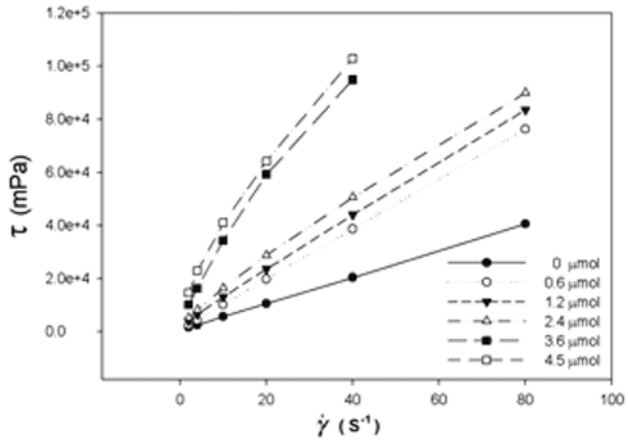

Figure 4. Behaviors of shear stress to strain rate for the chitin concentration in the blend solution.

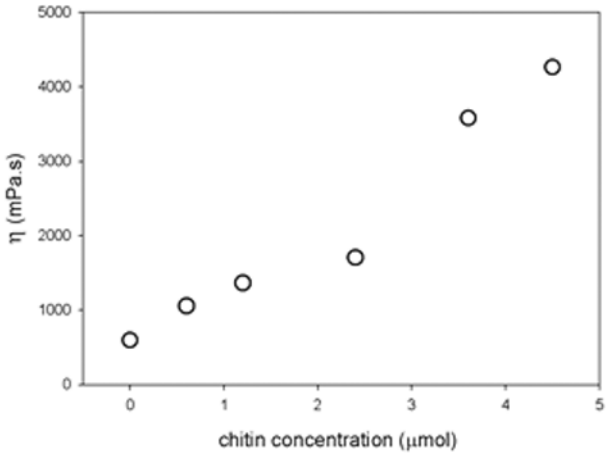

Figure 5. Variations of apparent viscosity to chitin concentration in the blend solution.

stresses of the solutions increased twice over by blending chitin. The viscosities of the blended solutions corresponding to slopes of the graphs were nearly constant up to 2.4 μmol of chitin concentration. Above this chitin concentration, viscosities increased rapidly from early stage and its increment decreased gradually, showing viscous behavior of pseudoplastic fluids at 3.6 μmol of chitin concentration and up. Figure 5 shows the apparent viscosity variation according to chitin concentration of the blend solution. The PDLLA/chitin blend solution showed a proportionately large increase of viscosity after the addition of relatively small amounts of chitin. This indicated that the extended rigid chains of chitin were tangled with flexible PDLLA chains, resulting in a great increase in the chain entanglement of the blend solution.

Morphology. Figure 6 showed the morphologies of a nanofiber web of PDLLA homopolymer which were electrospun based on the PDLLA concentration of dope under optimum spinning conditions; electric intensity 1.2 kV/cm, spinning speed 2 μL/min, and target speed 35 rpm. With a low polymer concentration of 15%, a bead-type webs with connected thin fibers was formed. Knotted nanofibers were formed at a polymer concentration of 20%. Smooth and thick nanofibers were formed at a polymer concentration of 25%. At a high concentration of 30%, uneven fibers were formed by gelation of dope. This was because the polymer concentration governs the viscosity of dope and the spinning property. The homogeneous solution of the PDLLA/chitin blend was obtained by using a chitin-salt complex and co-solvent, which was able to eject normally, forming a jet-cone. The even membrane of nanofibers was webbed on the target. The polymer concentration of the dope was fixed at 15% in order to conform to the spinning viscosity. The dope was ejected by electric intensity 1.6 kV/cm. The fabrication behaviors of nanofiber webs were examined depending on the chitin content of the PDLLA/chitin blend, and are shown in Figure 7. The average diameters of the blended nanofibers were nearly constant at about 150 nm, which was finer than that of about 600 nm diameter of the PDLLA nanofiber. This was because incorporation of chitin increased the dope viscosity to more than two times, as shown in pre-

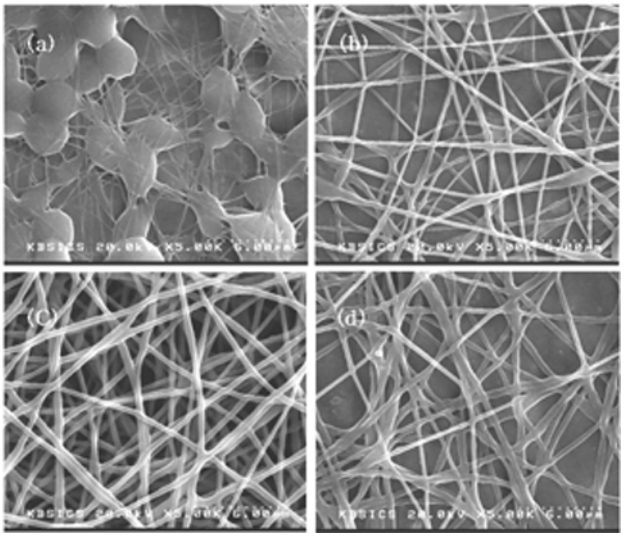

Figure 6. SEM images of the PDLLA membranes electrospun with PDLLA concentrations of (a) 15%, (b) 20%, (c) 25%, and (d) 30%, at a magnification of ×5,000.

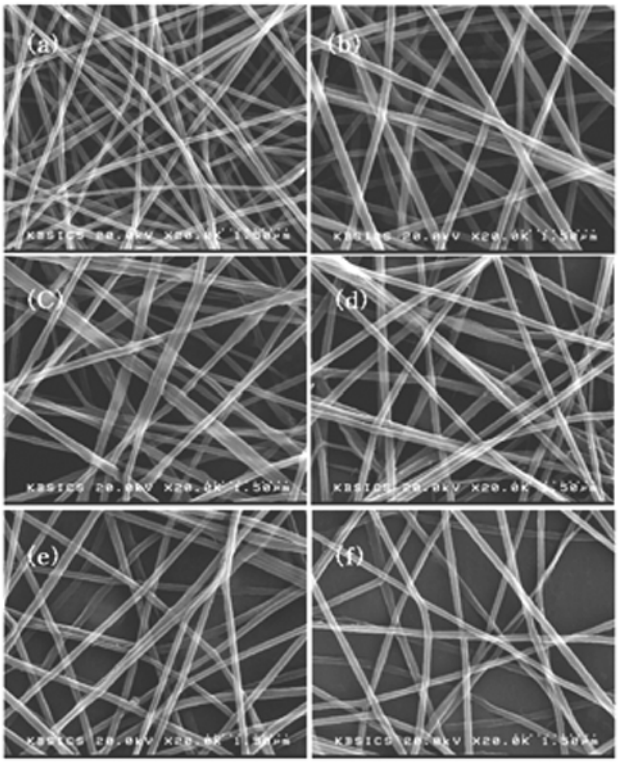

Figure 7. SEM images of the membranes of PDLLA/chitin blend with chitin concentration of (a) 0.6 μmol, (b) 1.2 μmol, (c) 2.4 μmol, (d) 3.6 μmol, (e) 4.5 μmol, and (f) 6.0 μmol at a magnification of ×20,000.

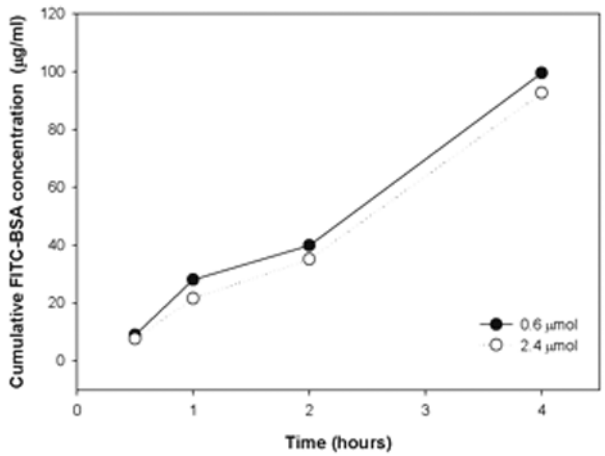

Figure 8. Permeative concentrations of FITC-BSA to the membranes with the chitin content of 0.6 μmol and 2.4 μmol as a function of elapsed time.

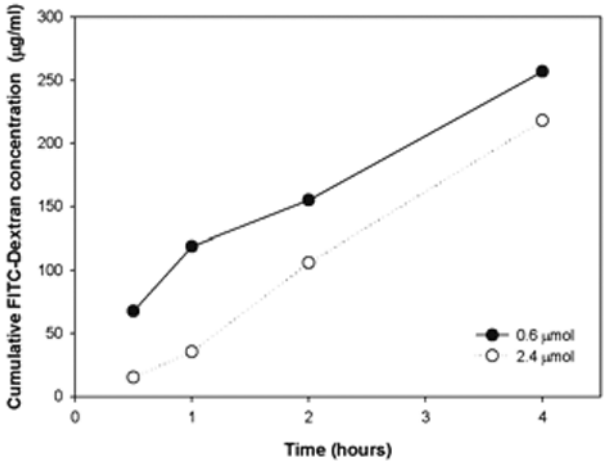

Figure 9. Permeative concentrations of FITC-Dextran to the membranes with the chitin content of 0.6 μmol and 2.4 μmol as a function of elapsed time.

vious result of viscosity. The salt complexed with chitin in dope might have contributed to the formation of even fibers because of an increase in the packing density of dope as in a research of the PDLLA/KH_2PO_4 system.[17] The average fiber spacing of the web membrane increased gradually from about 0.3 to 1 μm according to the increase in chitin content. The fabrication of the nanofiber web was difficult for the dope blended over the chitin content of 6 μmol. The increase of viscosity led to an increase in the interaction and the entanglement of neighbor polymer chains in the dope. Therefore excessive molecular entanglement caused a heterogeneous gelation in flowing dope, disturbing the uniform flow in its pathway and normal formation of jet cone in the spinning line.

Permeability. The permeation of biologically active compounds, including various growth factors and nutrients, are an important consideration for the successful application of GTR membranes as functional barriers. The permeability of nanofiber-webbing membrane (thickness 40 μm) of PDLLA/chitin blend was tested using FITC-BSA and FITC-Dextran as the model protein and model polysaccharide. Figure 8 and Figure 9 showed cumulative permeation concentrations versus the elapsed time for FITC-BSA and FITC-Dextran, respectively. The permeability could be controlled by adjusting the thickness of the web membrane, which was adequate for its use as a GTR membrane. There was a little gap between the permeation concentrations of FITC-BSA despite the difference of chitin content in the membranes. The permeation concentration of FITC-Dextran had more than doubled in comparison to that of FITC-BSA and showed an appreciable difference depending on the chitin content. The difference of permeation concentration between the model compounds may have been mainly attributable to the difference in their molecular weights; 66,000 vs 4,000. The decrement of permeation concentration depending on chitin content indicated that dextran of polysaccharide was more influenced by chitin of membrane than albumin of protein, and was better held on chitin that is similar in molecular characteristic.

Conclusions

Nanofibrous membranes made from blends of PDLLA and chitin, were prepared for GTR barriers using the novel solution blend and electrospinning. The blend of PDLLA and chitin-LiCl complex was formed a miscible NMP solution sufficient for electrospinning with the range of a given blend ratio. The viscosity of the blend solution significantly increased in a low ratio of chitin to the blend. The thinner nanofiber thereby was obtained in the blend than that in the PDLLAhomopolymer. The blending of chitin in the PDLLA matrix triggered the interactions between two polymeric components and also an ordered microstructure in electrospinning process of nanofibers. The membranes of the blend had a controlled permeability for bioactive model compounds.

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