Degradation of Electrospun Poly(L-lactide) Membranes Under Cyclic Loading

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ABSTRACT: Because of the great effect of mechanical loads on degradation of biodegradable biomaterials, investigation on degradation of electrospun membranes under dynamic loading is of importance. In this article, degradation of electrospun poly(L-lactide) (PLLA) membranes was carried out in a Tris-HCl buffer solution containing proteinase K under a cyclic stretch loading (dynamic condition) in comparison with that in shaking water bath (static condition). The evolutions of mass loss, morphology, thermal properties, and relative molecular weight of PLLA were monitored during degradation. The results showed that after degradation for 10 h, the mass of the electrospun PLLA membranes dropped by 38.7% and 31.3%, respectively, under dynamic and static conditions. And the electrospun PLLA membranes showed higher value of crystallinity during degradation under both cyclic loading and static condition. In addition, benzyl triethyl ammonium chloride (BTEAC) was introduced during electrospinning, and the effects of electrospun membranes characteristic and crystallinity on degradation were also investigated. The degradation study results are expected to be a basic study in mimicking actually mechanical environments. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 124: E258–E266, 2012

Key words: degradation; poly(L-lactide); electrospun membrane; cyclic loading

INTRODUCTION

Electrospinning facilitates intensive applications of polymers due to the versatile characteristics of electrospun membranes.[1–4] More specifically, electrospinning affords an opportunity to engineer scaffolds with micro- to nano-scale topography and high porosity similar to the natural extracellular matrix (ECM),[5] which enhances cell attachment,[6] drug load,[7] and mass transfer properties.[8] Whenever electrospun membranes are used for tissue engineering, effects of mechanical load should be taken into account. For one thing, most polymer implants inevitably endure mechanical loads in biological environments *in vivo*; for another, responses of the implants to dynamic loads cannot be easily predicted from experimental data by performing more traditional static testing.[9] Actually, mechanical analyses done to bones have drawn much attention,[10,11] whilst investigations on degradation and controlled drug release for tissue engineering under dynamic load are in progress.[12–14]

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Langer[15] addressed that nonstatic conditions favored chondrocyte growth and cartilage regeneration due to efficient mass transfer and controlled shear rates at the cell and implant surfaces. Tensile load, compressive load, and tensile-compressive combined loads were exerted on poly(D,L-lactic acid) foams, respectively, in Fan's research.[16] The experiments were carried out under constant stress, and the results showed that scaffolds under continuous load degraded obviously faster than those free from loads. In early study, we probed into degradation of porous poly(L-lactide-*co*-glycolide)/β-tricalcium phosphate (PLGA/β-TCP) and PLGA scaffolds, respectively, and dynamic compressive load was customized with frequency of 1 Hz and locomotion of 0.50–0.60 mm.[17,18]

To imitate mechanical load the implants undergo in biological environment, dynamic loads appear to be more reasonable. It is documented that quite broad frequency range of mechanical load is applied to bone during normal locomotion.[19] Apart from the dynamic compressive load, dynamic stretch load is also of importance for the scaffolds. However, few of the publications address the effect of dynamic stretch load on scaffolds.

As bioabsorbable polymers, poly(L-lactide) (PLLA) and its copolymers are ideal candidates for tissue engineering,[20,21] and have attracted the most attention for surgical repair purposes.[22] Electrospun

PLLA membrane is suitable for experiencing stretching load for degradation studies due to the unique status. Because morphology of electrospun fibers strongly depends on the solution properties in electrospinning,[23] benzyl triethyl ammonium chloride (BTEAC, quaternary ammonium salt) is introduced to fabricate electrospun PLLA membrane containing BTEAC (PLLA/BTEAC). BTEAC has been used to adjust the core/shell electrospun ultrafine fibers of PVDF/PC in our previous study.[24] In this paper, effect of electrospun PLLA/BTEAC membrane characteristic on degradation was particularly studied.

A short degradation term is required for applying cyclic stretch loading more conveniently, and proteinase K makes it possible to study degradability of electrospun PLLA membranes in a curtailed time span. A Tris-HCl buffer solution (pH 8.6) with proteinase K (Tris-HCl/proteinase K) was used in this work for incubation with PLLA and PLLA/BTEAC electrospun membranes. Proteinase K is an endoprotease and favors cleavage of peptide bonds C-terminal to both aliphatic and aromatic amino acids as well as alanine.[25] Since the chemical structures of lactic acid and alanine are alike in some aspects, proteinase K can be employed to accelerate the hydrolysis of poly(L-lactide).

The aim of this research is to address preliminary results for devoting the biodegradable electrospun membranes to a more actual environment for more practical applications. Cyclic stretch loadings were applied to electrospun PLLA and PLLA/BTEAC membranes incubating in Tris-HCl/proteinase buffer solution (dynamic condition), respectively. Degradation of electrospun membranes free from cyclic loading was also performed (static condition) for comparison. The degradation process was monitored by investigating mass loss, morphology, thermal properties, and molecular weight of PLLA as well as pH value in buffer solution. The effect of electrospun membrane characteristic on degradation was another interest for this article.

EXPERIMENTAL

Materials

PLLA (viscosity–average molecular weight 1.01×10^5) was kindly supplied by Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, China, and used after purification. BTEAC was provided by Sigma-Aldrich. Proteinase K (lyophilized powder, 30 mAnson-U/mg) was purchased from Merck and used as received. Tris-hydroxymethylaminomethane (Tris) was also obtained from Merck and used for preparation of Tris-HCl buffer solution. Dichloromethane (DCM), *N*,*N*-dimethylformamide (DMF), and chloroform were all of analytical grade and used without further purification.

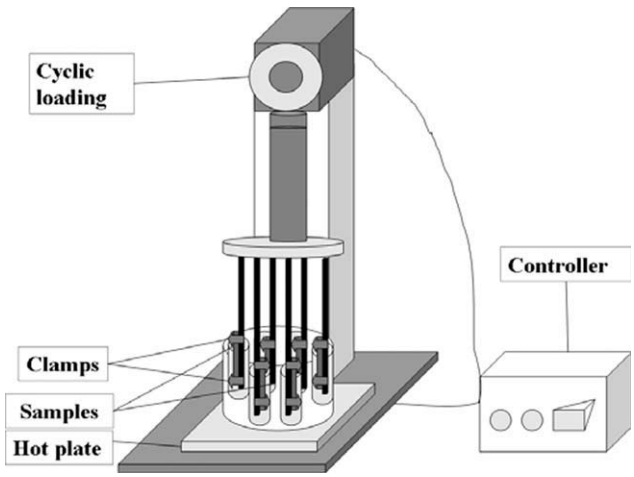

Figure 1 Cyclic stretch apparatus for degradation.

Electrospinning

The purified PLLA was dissolved in a DCM/DMF mixed solvent (70/30, v/v) to prepare PLLA solution with a concentration of 0.08 g/mL. PLLA/BTEAC solution was fabricated in the same way with the addition of BTEAC (4 wt% of PLLA).

Electrospinning setup composed of a syringe pump (Cole-Parmer Instrument, USA), a grounded rotating drum wrapped with aluminum foil, and a high voltage supply (MGD1-A, Tianjin Dongwen High Power Voltage Supply, Tianjin, China) was shown in our previous study.[26] The PLLA (or PLLA/BTEAC) solution was electrospun at applied voltage of 12 kV with 0.6 mL/h solution flow rate, and the capillary-collector distance was set as 15 cm. Electrospun membranes were then dried in a *vacuo* at room temperature for 48 h and stored at 4°C for degradation studies.

Degradation studies

Tris-HCl buffer solution with a concentration of 0.05 mol/L containing 5 μg/mL proteinase K was prepared, and was modulated to pH 8.6 with dilute HCl. Electrospun membranes were cut into strips (4 cm × 1 cm, about 25 mg), and the two ends of a strip were fixed in the upper and the lower clamps, respectively, in the cyclic stretch loading apparatus as shown in Figure 1. All the strips were ensured to be immersed in the containers, which were filled with the buffer solution at 37.0 ± 0.1°C and covered with lids. Cyclic stretch loading was customized with frequency of 1 Hz and load locomotion of 0.60 mm for degradation studies of electrospun membranes. Six paralleled samples were removed from

the buffer solution at appropriate time intervals (2, 4, 6, 8, and 10 h), and pH values of the incubated buffer solution were measured by a pH meter (Delta 320, Mettler-Toledo). Electrospun membranes after degradation were washed thoroughly with distilled water, and then vacuum-dried at least 48 h. The remaining mass of electrospun specimen was measured accurately, and calculated mass loss was given by:

$$\text{Mass loss } (\%) = (m_0 - m_d)/m_0 \times 100\% \quad (1)$$

where m_d represents the mass of vacuum-dried specimen after degradation, while m_0 is the initial mass of the specimen.

Degradation free from cyclic loading for electrospun membranes was also performed as control. Specimens were incubated with Tris-HCl/proteinase K buffer solution in vials sealed with Teflon, and the vials were moored in shaking water bath (37.0 ± 0.1°C) for degradation.

Characterization method

Original electrospun membranes were coated with gold for observation under a scanning electron microscope (SEM, Philips XL-30), and average diameters for electrospun membranes were assessed from SEM micrographs with Photoshop software.

Morphological evolution of electrospun membranes during degradation was also observed under SEM. DSC measurement was performed on a Perkin Elmer Diamond apparatus under nitrogen atmosphere using sample of about 5 mg. The sample was heated up to 200°C, and then quenched to room temperature. The second heating run was scanned again to 200°C at a rate of 10°C/min, and the trace was recorded. The electrospun membrane after degradation was dissolved in chloroform, and the intrinsic viscosity was determined by an Ubbelohde viscometer in a thermostat at 25.0±0.1°C with a concentration of 1 mg/mL. Viscosity–average molecular weight of PLLA can be obtained with Mark-Houwink equation.

RESULTS AND DISCUSSION

Characterization of electrospun membranes

SEM micrographs of original PLLA and PLLA/BTEAC electrospun membranes are shown in Figure 2. Smooth PLLA ultrafine fibers were obtained, and some were fused at junctions. The diameters of PLLA fibers were in the range of 300–450 nm with an average of 390 ± 11 nm. The average diameter for electrospun PLLA/BTEAC fibers was 262 ± 9 nm, which mainly distributed at 150–350 nm. It is believed that the electrospum fiber morphology is apparently affected by the properties of solution in electrospinning.[27] The introduction of BTEAC increased the conductivity and depressed the surface tension of the solution, which rendered enforced net charge density on the fluid jet during electrospinning. Therefore, the addition of BTEAC effectively reduced the fiber diameters in electrospun membranes.

Degradation of electrospun membranes

The reason for choosing 10 h as the terminal for degradation study is that some of the membranes collapse around 10 h under cyclic stretch loading, and the specimens could not be tested adequately. For comparison, degradation of electrospun membranes free from cyclic loading was also examined within 10 h.

Mass loss

Figure 3 illustrates mass loss of electrospun PLLA and PLLA/BTEAC membranes during degradation. The values of mass loss of all the electrospun

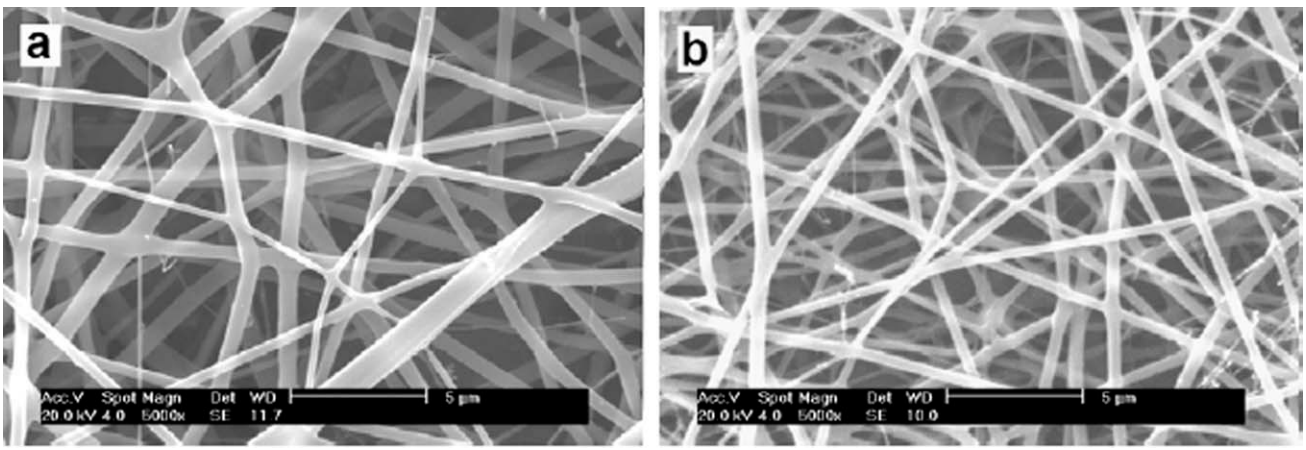

Figure 2 SEM micrographs of electrospun membranes: (a) PLLA, (b) PLLA/BTEAC.

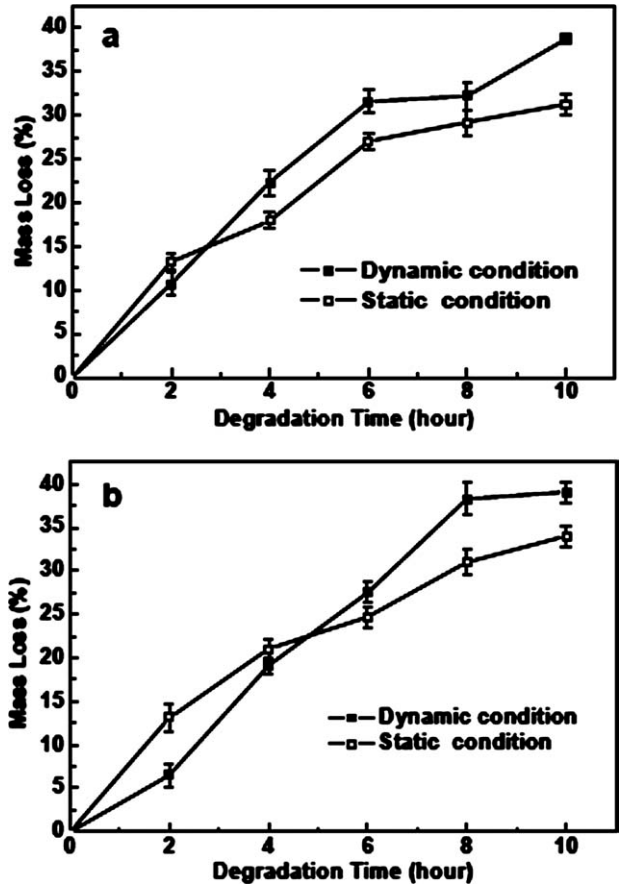

Figure 3 Mass loss of electrospun membranes during degradation: (a) PLLA, (b) PLLA/BTEAC.

membranes increased with the degradation time for both dynamic and static conditions. The variations of mass loss indicate that degradation rates are almost the same for the two series of electrospun membranes [Fig. 3(a,b)], but show differences in diverse time ranges.

Electrospun PLLA membranes lost straightly about 31.5% and 27.0% of the initial mass in 6 h under dynamic and static condition, respectively. Then the degradation exhibited slower rate, and the values of mass loss after 10 h were about 38.7% and 31.3% for dynamic and static condition, respectively. As for the electrospun PLLA/BTEAC membranes, mass losses increased drastically to about 38.2% and 31.0% after degradation of 8 h under dynamic and static conditions, respectively. Then the mass losses experienced slight rise, and reached about 39.1% and 34.0% in 10 h for dynamic and static condition, respectively.

Note that there are intersections in mass loss delineations between dynamic and static conditions. The crossings in plots indicate that the mass loss under dynamic condition is not significant in the early period of degradation. As time was prolonged, degradation under dynamic condition speeded up exceedingly, and more apparent discrepancy of mass loss between dynamic and static condition was notable. In this study, 10 h is adopted for degradation because of collapse of specimens, and what the time span brings about is that degradation has not been fulfilled to intensive extent. Consequently, the discrepancy of mass loss between dynamic and static condition is seemingly not significant. The effect of cyclic loading on degradation is approved in following discussion notwithstanding mass loss.

Rapid degradation under mechanical load has been published in Fan's early indication.[16] Mechanical load can promote the alterations of covalent bonds in length or angle, and could further affect the stability of covalent bonds in polymers. Cyclic stretch loading in this research facilitates the mobility and rearrangement of segments, which benefits the degradation of PLLA. Besides, better flowing under dynamic load promotes buffer solution penetrating inside the substrates, and proteinase K can attack PLLA chains in the amorphous region preferentially.[28] In the early stage of degradation, cyclic loading is not forceful enough to change the covalent bonds in PLLA chains. As degradation proceed,

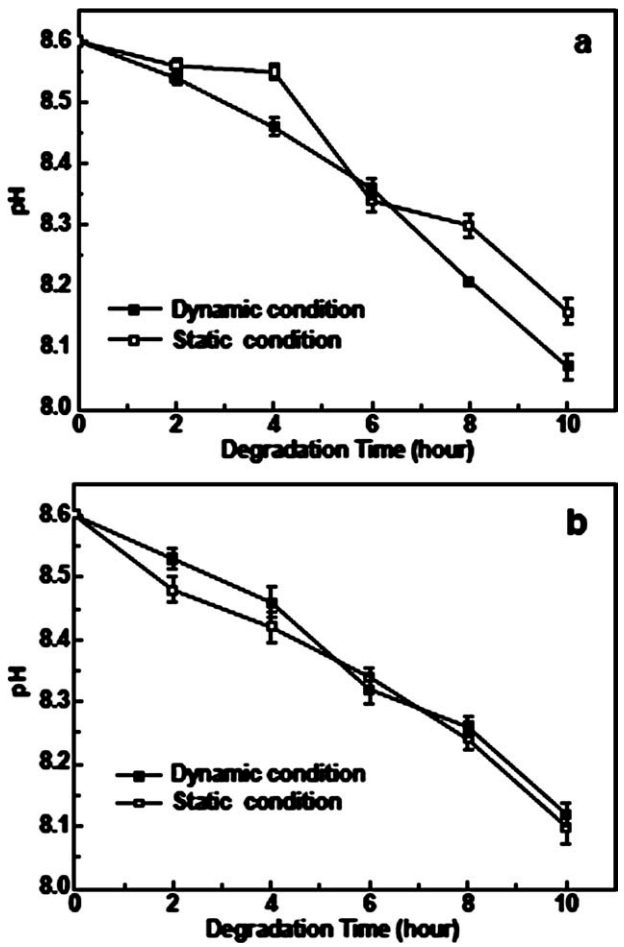

Figure 4 Variations of pH for buffer solution during degradation of electrospun membranes: (a) PLLA, (b) PLLA/BTEAC.

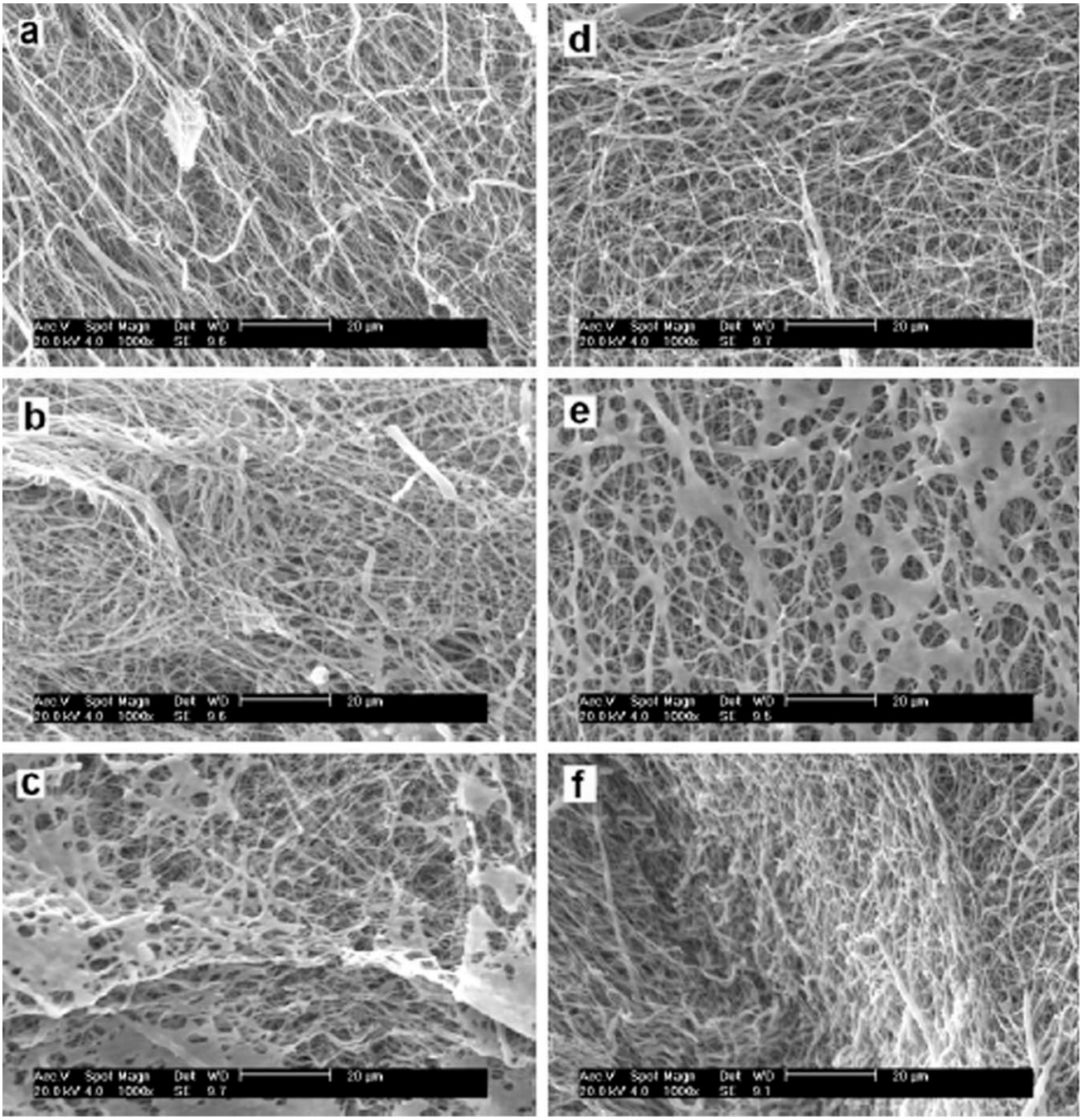

Figure 5 SEM micrographs of electrospun PLLA membranes after degradation: (a) dynamic condition 2 h, (b) dynamic condition 6 h, (c) dynamic condition 10 h, (d) static condition 2 h, (e) static condition 6 h, and (f) static condition 10 h.

the cyclic loading operated, and accelerated degradation was achieved by both dynamic mechanical load and enzyme.

pH value

Variations of pH values for buffer solution during degradation are delineated in Figure 4. All the pH values decreased straightforward, and reached at about 8.1 after incubation with buffer solution for 10 h. The drop of pH indicated the cleavage of ester bonds and the emergence of compounds with lower molecular weight.

The pH value for optimum activity of proteinase K ranges from 7.5 to 12.0.[29] In this research, all the pH values lie over 8.1, and the bioactivity of proteinase K is ensured. The variations of pH values also indicated the effect of electrospun membrane characteristic, which was clarified as follows. The pH values under static condition were clearly higher most of the time than those under dynamic stretching load for electrospun PLLA membranes, and the

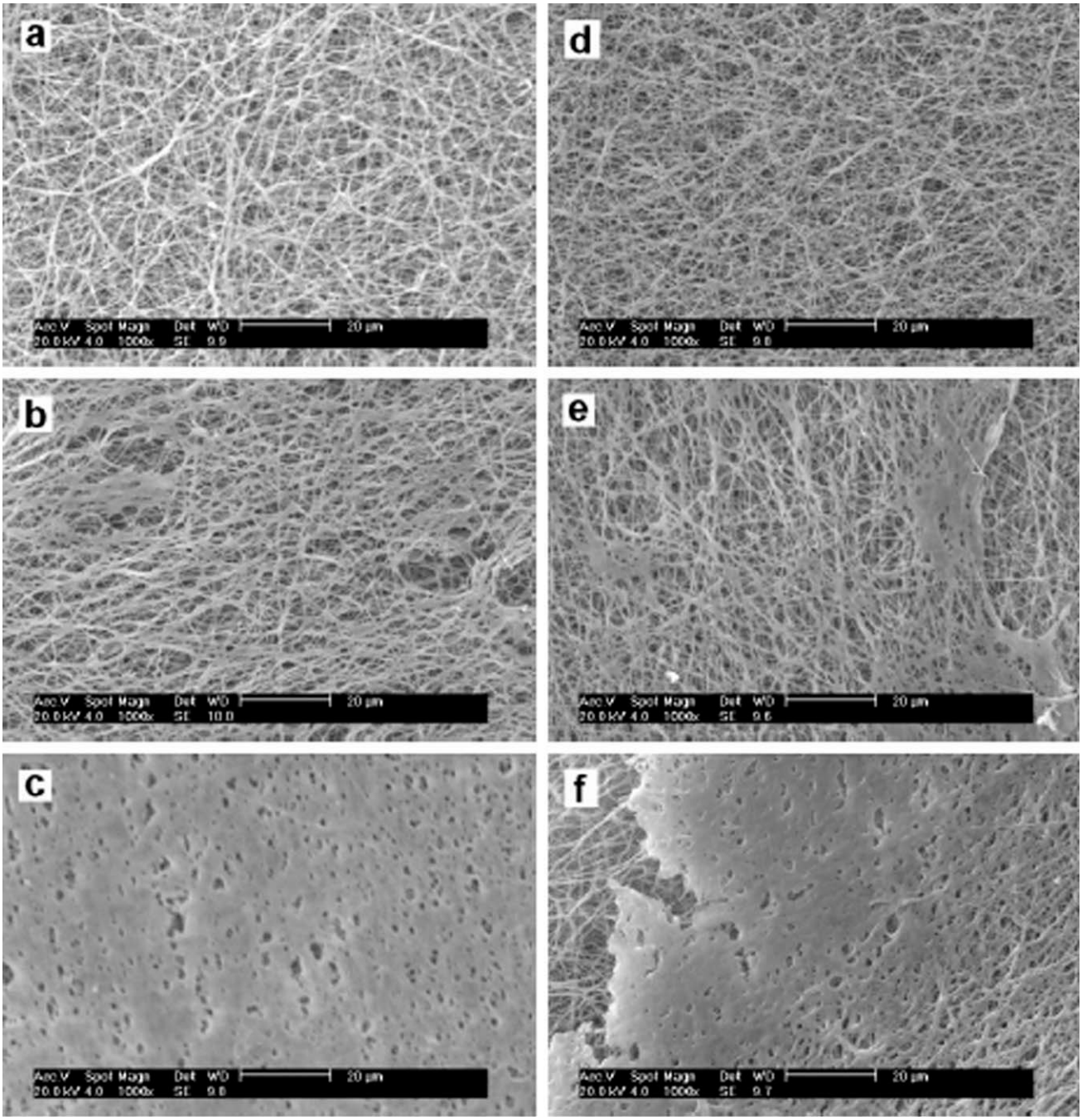

Figure 6 SEM micrographs of electrospun PLLA/BTEAC membranes after degradation: (a) dynamic condition 2 h, (b) dynamic condition 6 h, (c) dynamic condition 10 h, (d) static condition 2 h, (e) static condition 6 h, and (f) static condition 10 h.

discrepancy of pH values between dynamic and static condition was obvious. However, in the degradation of electrospun PLLA/BTEAC membranes, cyclic stretch loading seemed not lead to distinct decline of pH value in comparison with those free from cyclic loading. Apart from the degradation rate, the leaching of the acidic molecules from the electrospun membranes plays an important role for pH values. Better flowing of buffer solution under dynamic condition favors the release of soluble acidic molecules from the electrospun membranes, and pH values in buffer solution decrease notably. The higher surface to volume ratio of electrospun PLLA/BTEAC fibers (Fig. 2) is supposed to interact with proteinase K and degrade significantly, and the leaching of acidic molecules is also easily either in dynamic or static condition. Moreover, we can find in the DSC measurement (see DSC analysis) that electrospun PLLA/BTEAC fibers exhibited lower crystallinity than electrospun PLLA fibers, and were prone to degrade. The aforementioned are all contributed to the slight discrepancy of pH value in

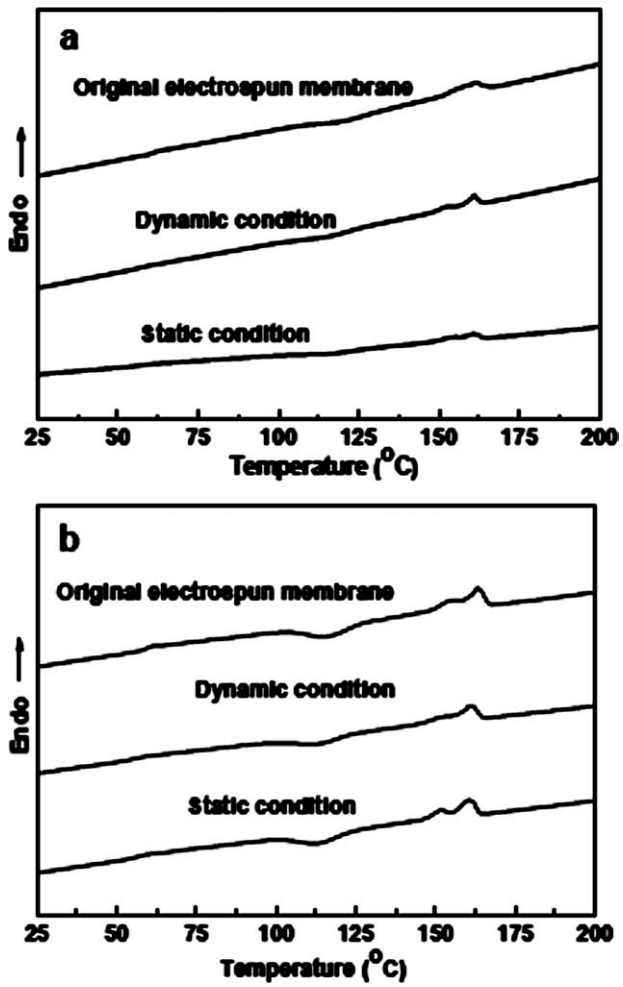

Figure 7 DSC traces for original electrospun membranes and specimens after degradation for 10 h: (a) PLLA, (b) PLLA/BTEAC.

electrospun PLLA/BTEAC membrane degradation between dynamic and static conditions [Fig. 4(b)].

Morphology

Figures 5 and 6 compile SEM micrographs of electrospun membranes during degradation, in which rough fiber surfaces are detected.

It is hard for hydrophilic enzyme to penetrate into hydrophobic substrate,[30] and enzyme could only act on PLLA fibers from the surface to the inside. The rough surfaces of fibers were mainly due to the degradation of PLLA.

Fibers in electrospun PLLA membranes sustained their structures at the degradation time of 2 and 6 h except for some fused together. The degradation under cyclic stretch loading was proceed to a certain extent at 10 h, and most of the fibers collapsed with agglomerations in the residue [Fig. 5(c)]. Obvious effect of cyclic loading was brought to the degradation of thinner fibers in electrospun PLLA/BTEAC membranes. In the initial 2 h, morphological changes were observed with rough fiber surfaces, while some of the fibers fused at the junctions after degraded 6 h. Seriously agglomerated chunks were witnessed for samples degraded under dynamic condition for 10 h [Fig. 6(c)].

The visible observations of morphology agreed well with the variation results of mass loss and pH value, which inferred that mechanical load, electrospun membrane characteristic, and crystallinity all have effects on the degradation behavior.

DSC analysis

Figure 7 presents DSC traces for starting electrospun membranes and samples after degradation of 10 h. Summary of characteristic data, namely, melting temperature (T_m, taken from the endothermic peak) and its enthalpy ($\triangle H_m$), cold crystallization temperature (T_{cc}) and related enthalpy ($\triangle H_{cc}$), glass transition temperature (T_g), and crystallinity (X_c) are collected in Table I (Ref. [31] is also included). Starting electrospun PLLA membrane showed T_g at 60.9°C and a main melting peak appeared at 161.1°C, which is somewhat agrees well with the research results of PLLA electrospun membranes.[32,33] Characteristic thermal properties for original electrospun PLLA/BTEAC membranes were estimated with T_g at 59.0°C and melting temperature at 163.1°C.

In evaluation of crystallinity for samples, values of $\triangle H_{cc}$ were subtracted from initial melting enthalpy, while the melting enthalpy for PLLA/BTEAC are calibrated according to the actual mass of PLLA in electrospun membrane as follows: [34]

$$\Delta H_m = \frac{\Delta H_0}{1 - x} \tag{2}$$

TABLE I
Characteristic Data of DSC for Original Electrospun Membranes and Specimens After Degradation for 10 h

	PLLA						PLLA/BTEAC					
Sample	T_g (°C)	T_{cc} (°C)	H_{cc} (J/g)	T_m (°C)	H_m (J/g)	X_c[a] (%)	T_g (°C)	T_{cc} (°C)	H_{cc} (J/g)	T_m (°C)	H_m (J/g)	X_c[a] (%)
Electrospun membrane	60.9	120.2	11.2	161.1	24.2	9.6	59.0	115.5	16.9	163.1	27.6	8.3
Dynamic condition	56.7	116.4	11.4	160.9	27.8	12.1	55.6	112.7	14.7	160.4	28.4	10.6
Static condition	56.9	115.5	12.1	160.6	28.8	12.4	56.1	113.1	16.4	162.8	31.1	11.3

[a] $H_{m0} = 135$ J/g.[31]

where $\triangle H_0$ is the original melting enthalpy obtained from DSC analysis, and x is the weight fraction of BTEAC in samples.

Electrospun PLLA/BTEAC membranes exhibited higher melting enthalpy (27.6 J/g) than that of PLLA (24.2 J/g). It is evident that the addition of BTEAC increased and charge density in ejected jet, and self-repulsion of the excess charges imposed stronger elongation forces on jet under the electrical field.[35] The extension strength in electrospinning was attributed to the higher melting enthalpy for electrospun PLLA/BTEAC membranes. However, the enthalpy of cold crystallization for electrospun PLLA/BTEAC membranes is significant, and results in the actual decreased crystallinity.

Note that the values of T_g for both electrospun PLLA membranes and PLLA/BTEAC membranes dropped after degradation. The breakage of chains in degradation promotes the mobility and rearrangement of segments, which induces the decrease of T_g for polyesters.

Electrospun PLLA membrane showed increased crystallinity of 12.1% and 12.4%, respectively, for dynamic and static condition after 10 h of degradation, and the corresponding rise rates of crystallinity were 26.0% and 29.2%. Crystallinities for electrospun PLLA/BTEAC membranes increased to 10.6% and 11.3%, respectively, after degradation under dynamic and static conditions for 10 h, and the related increase rates were 27.7% and 36.1%. It is not surprising to find that electrospun PLLA/BTEAC membranes with lower initial crystallinity showed a quicker rise in crystallinity during degradation, while electrospun PLLA membranes with relatively higher initial crystallinity exhibited lower increase rate of crystallinity. The evolution of crystallinity demonstrated the cleavage-induced crystallization mechanism. Hsiao et al.[36] proposed that the cleavage of amorphous chains encouraged mobility to form new crystal lamellar stacks when degradation occurs in the amorphous gaps between crystal lamellar stacks. Moreover, degradation can proceed through the interlamellar amorphous layer between the adjacent lamellae. The research also suggested that post microstructure developed by cleavage-induced crystallization slightly hindered degradation process, which is coincided with the variations of mass loss in our present study.

Average molecular weight

Estimates of viscosity–average molecular weight have been done to electrospun PLLA membranes during degradation, and the results are presented in Figure 8.

The viscosity–average molecular weight of PLLA exhibited nearly linear relationships with the degradation time in semilog plot. The slight decreases in viscosity–average molecular weight showed roughly similar extents under dynamic and static condition.

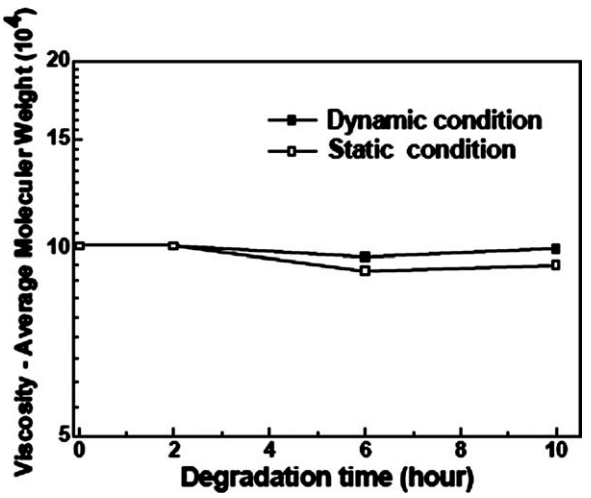

Figure 8 Viscosity–average molecular weight of PLLA during degradation.

The release of soluble compound with lower molecular weight may result in a slight increase of molecular weight for PLLA substrate, while the scission of ester linkage backbone may cause the drop of PLLA molecular weight. Therefore, the resulting molecular weight of PLLA depends on degradation mechanism it followed. Degradation of electrospun PLLA membranes under static condition is mainly dominated by enzymatic degradation, which would like to behave as some kind of surface erosion. But the variations of viscosity–average molecular weight did not appear to be the typical surface degradation as indicated in reference.[37] The reason is probably due to the degradation time span adopted in this research, in which the degradation has not been fulfilled to intensive extent. McCarthy[38] had concluded in the early research that degradation of PLA stereocopolymer by proteinase K depended on many factors (such as crystallinity, stereochemistry, and hydrophilicity). The present accelerated degradation of electrospun PLLA membranes under cyclic loading incubating in proteinase K is likewise complex. Returning again to consider the results, loading, electrospun membrane characteristic, and crystallinity are all of importance for degradation. Further studies are in progress to elucidate the mechanism of the degradation process potentially.

CONCLUSION

Electrospun PLLA and PLLA/BTEAC membranes were subjected to degradation in Tris-HCl/proteinase K buffer solution under dynamic and static condition, respectively. The results showed that accelerated degradation of electrospun PLLA membranes was facilitated by cyclic stretch loading in addition

to proteinase K. Cyclic loading did not significantly affect the degradation in the early period, but promoted degradation in the following stage. The discrepancy of pH value between dynamic and static condition is more significant for electrospun PLLA membranes. Obvious effect of cyclic loading on morphology was brought to electrospun PLLA/BTEAC membranes. The results of DSC suggested the addition of BTEAC was vital for crystallinity of the electrospun membranes, and the cleavage-induced crystallization mechanism was reasonable. PLLA showed trivial variation in viscosity–average molecular weight in the degradation time range, which is attributed to the degradation mechanism it follows.

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