Synthesis and Characterization of New Biodegradable Polymers for Biomodeling and Biomedical Applications

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Summary: Biodegradable poly(1,2-propanediol succinate) and poly(1,3-propanediol succinate) were synthesized using 1,2-propanediol and 1,3-propanediol with succinic anhydride, respectively. The synthesized polymers were identified by NMR spectrometry and FT-IR spectrophotometry. The weight average molecular weights were 8,900 in poly(1,2-propanediol succinate) and 8,600 in poly(1,3-propanediol succinate), respectively. The biodegradation behavior of polymers in microorganisms was studied by using a modified ASTM method. These polyesters were degraded to lower molecular weight compounds depending on hydrophobicity and hydrophilicity. The quantitative determination of carbon dioxide, generated during the treatment with the activated sludge, showed that poly(1,3-propanediol succinate) was biodegraded faster than poly(1,2-propanediol succinate). The biodegradation ability of the polymers by *Aspergillus niger* was monitored to check the molecular weights using GPC and to check the crystallinity change of the polymers using DSC. Based upon the visual observation of the biodegraded polymers, the crystalline structure of poly(1,2-propanediol succinate) apparently retained its crystallinity longer than the similar structure in poly(1,3-propanediol succinate).

Keywords: biodegradable; biomaterials; crystallization; hydrophobicity; morphology

Introduction

Biodegradable polymers have mainly been used in the development of polymeric matrices for the controlled and sustained release of low molecular weight therapeutic agents and short-term implants such as sutures and surgical staples for biomedical applications. Such implants should maintain their functionality over a relatively short period of time. However, efforts to develop resorbable implants that will fulfill more demanding functions, such as vascular prosthesis and osteosynthesis devices (bone screws, plates, pins, or intramedullary nails) have been recently continued. Such implants must maintain chemical and mechanical stability *in vivo* over a sufficient period of time for the fulfillment of their

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primary function of allowing regeneration of the substituted organ or assuring adequate stability of bone fracture during healing. The selected medical applications of resorbable polymers have been addressed in various papers.[1-5] The scarcity of polymers that meet these rather demanding requirements has prompted a continuous search for improved biodegradable polymers. One of the advantages of chemically synthesized biodegradable polymers is that their degradation rates can be controlled at will, so as to meet specific requirements by chemical modification of the structures. There are many factors affecting the biodegradability of polymeric materials. They are related to primary structure (chemical composition, molecular weight, molecular weight distribution, etc.), higher-order structure (melting point, glass transition temperature, crystallinity, crystal structure, etc.), and surface conditions (surface area, hydrophilicity, hydrophobicity, etc.).[6,7] Since these factors are interrelated in a complicated way, it is not easy to clarify the structure--biodegradability relationships for a wide range of polymers. In our laboratory, efforts have been recently directed toward the synthesis of new biodegradable polymers for biomodeling and biomedical applications.[8-12] New monomers such as 1,4-butanediol dilactate and 2-acetoxy succinic acid were synthesized for the development of new biodegradable polymers. Additionally, new biodegradable polymers such as poly(1,4-butanediol succinate), crosslinked poly(1,4-butanediol L-malate), and crosslinked poly(glycerol-co-malate) were synthesized using Krebs cycle acid derivatives. The hydrolytic behaviors in various pH buffer solutions and biodegradation by microorganisms were studied for the synthesized polymers. Thus far, the effects of crystallinity and molecular weights on the biodegradation have also been investigated.

In the present work, poly(1,3-propanediol succinate) and poly(1,2-propanediol succinate) containing a pendant hydrophobic methyl group were synthesized using 1,3-propanediol and 1,2-propanediol, respectively, with Krebs cycle acid derivatives such as succinic anhydride. The effects of the pendant hydrophobic group and crystallinity were investigated on the poly(1,2-propanediol succinate) and poly(1,3-propanediol succinate). Their morphology changes during biodegradation were observed using optical microscopy to compare their biodegradabilities.

Experimental

Materials and synthesis. Succinic anhydride, 1,2-propanediol, and 1,3-propanediol were purchased from Aldrich Chemical Co., and *p*-toluenesulfonic acid, which was used as a

catalyst, was obtained from the Sigma Co. Succinic anhydride, 1,3-propanediol, and *p*-toluenesulfonic acid were dissolved in toluene and placed in a two-necked flask equipped with a magnetic stirring bar, thermometer, and dean-stark trap on top of which is a condenser fitted with a drying tube. The resulting mixture was stirred and heated under reflex for 24 hours. The reaction mixture was filtered and washed with acetone. The product was recrystallized with chloroform and diethyl ether and dried in vacuum at room temperature for one week. Poly(1,2-propanediol succinate)(1,2-PPS) was polymerized using succinic anhydride and 1,2-propanediol for 96 hours using the same method as poly(1,3-propanediol succinate)(1,3-PPS).

Characterization. ^{13}C-NMR spectra were recorded with a Varian Gemini 200 spectrometer using $CDCl_3$ as a solvent. The FT-IR spectra in the solid state were recorded on a Perkin Elmer Spectrum One FT-IR spectrophotometer using a KBr pellet. Weight average molecular weights and number average molecular weights of the polymers were measured using a Shimadzu LC-10AD system. The columns were calibrated with polystyrene standards, having a narrow molecular weight distribution. The melting temperatures (T_m) of the polymers were measured with a differential scanning calorimeter (Perkin-Elmer DSC-7 system). The calibration of the temperature was performed using indium as a standard material.

Determination of carbon dioxide during biodegradation. The activated sludge was obtained from a sewage treatment plant. A sample of polymer (1.0 g) was charged in a flask containing the activated sludge (200 mL). Air from a pump was passed through a 0.05 *N* aqueous barium hydroxide solution in five flasks connected in series (No. 1 to 5), and the resulting carbon dioxide-free air was bubbled into the activated sludge at a constant flow rate of 25 mL/min at 27°C. Carbon dioxide generated during catabolism was quantitatively captured by passing the gas from the activated sludge through the 0.05 *N* aqueous barium hydroxide solution in five flasks. Then the sample was collected on a filter, dried, and weighed. There was no precipitation of barium carbonate in flasks such as No. 9 and No. 10. This result means that carbon dioxide was completely captured by the aqueous barium hydroxide solution in the three flasks No. 6 to 8. The amount of carbon dioxide evolved by catabolism was calculated from the weight of barium carbonate in a control run without sample from the total weight of barium carbonate.

Biodegradation. The microorganism for the biodegradation test was *Aspergillus niger*. *Aspergillus niger* is routinely used in the ASTM procedure for the determination of

biodegradation.[13] The conidia harvested from the 7 day culture slant were washed with distilled water three times. The harvested conidia were suspended in nutrient salt broth by 10^9 conidia/mL. The nutrient salt broth was prepared by dissolving in 1 L of water the designated amounts of the reagents in Table 1. The test medium was sterilized by autoclaving at 121°C for 20 min. The pH of the medium was adjusted by the addition of a 0.01 *N* NaOH solution so that after sterilization the pH was 6.2. Polymer disks, weighing 1.0 g and 5.0 x 13 mm in size, were prepared by compression molding the polymer powder at 5,000 kg for 3 min. using a hydraulic press (Graseby Specac Ltd.). The disks were placed in 100 mL of *Aspergillus niger*-nutrient salt broth solution at 28°C with constant orbital shaking at 75 rpm. The degradation residue was extracted using chloroform and ethyl ether. Polymer degradation was estimated from the molecular weight changes of samples by GPC and the enthalpy changes of degradation products were also measured with DSC.

Table 1. The preparation of nutrient-salt broth.

Reagents	Weight
Potassium dihydrogen orthophosphate (KH_2PO_4)	0.7
Magnesium sulfate ($MgSO_4$ $7H_2O$)	0.7
Ammonium nitrate (NH_4NO_3)	1.0
Sodium chloride (NaCl)	0.005
Ferrous sulfate ($FeSO_4$ $7H_2O$)	0.002
Zinc sulfate ($ZnSO_4$ $7H_2O$)	0.002
Manganous sulfate ($MnSO_4$ $7H_2O$)	0.001

Morphology changes. Acid-washed cover glasses for the microscope (18x18 mm) were flooded with 75μL of polymer-chloroform solution at a concentration of 0.16 g/mL. Solvent was evaporated on a hot plate and the polymer film was cooled slowly to room temperature. Less than 40 μm thick polymer film was deposited on the glass. The prepared film was sterilized at 140°C for 4 h in a dry oven and placed on an agar plate. The prepared suspension of each microorganism was inoculated on the polymer film surface and incubated at 28°C. The polymer film on the cover glass was removed from the incubator after a predetermined time and the shape was observed using optical microscopy (Leica DMLM microscope).

Results and Discussion

Identification. In the ^{13}C-NMR spectrum of poly(1,2-propanediol succinate), seven signals were shown carbon: (a) at 177ppm, carbon (b) at 31ppm, carbon (c) at 29ppm, carbon (d) at 172ppm, carbon (e) at 62ppm, carbon (f) at 16ppm, and carbon (g) at 77ppm, as shown in Figure 1. In the ^{13}C-NMR spectrum of poly(1,3-propanddiol succinate), six signals were shown: carbon (a) at 177ppm, carbon (b) at 30ppm, carbon (c) at 172ppm, carbon (d) at 62ppm, carbon (e) at 28ppm, and carbon (f) at 60ppm, as shown in Figure 2.

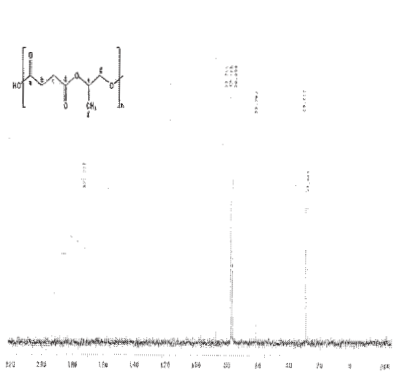

Figure 1. ^{13}C NMR spectrum of poly(1,2-propanediol succinate).

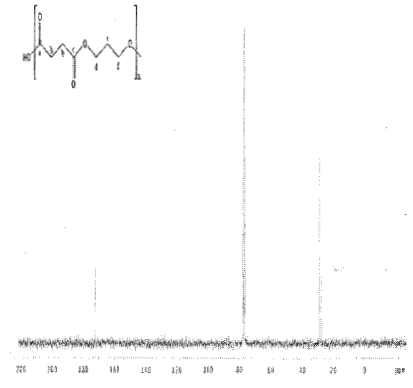

Figure 2. ^{13}C NMR spectrum of poly(1,3-propanediol succinate).

The FT-IR spectra of the 1,2-PPS and 1,3-PPS are shown in Figure 3. The IR vibrational band characteristic of ester bond formation is indicated by the presence of a carbonyl peak at 1,720 cm^{-1}. The M_w and M_n of poly(1,2-propanddiol succinate) were 8,900 and 5,400, respectively. The M_w and M_n of poly(1,3-propanediol succinate) were 8,600 and 5,030, respectively.

Biodegradation in activated sludge. The biodegradation of the polymer consists of two processes: (1) a degradation process, in which polymers are biodegraded to lower molecular weight compounds, and (2) a catabolism process, in which the lower molecular weight compounds are converted to water, carbon dioxide, etc. Direct and confirmative evidences for biodegradation can be obtained by quantitative analysis of carbon dioxide

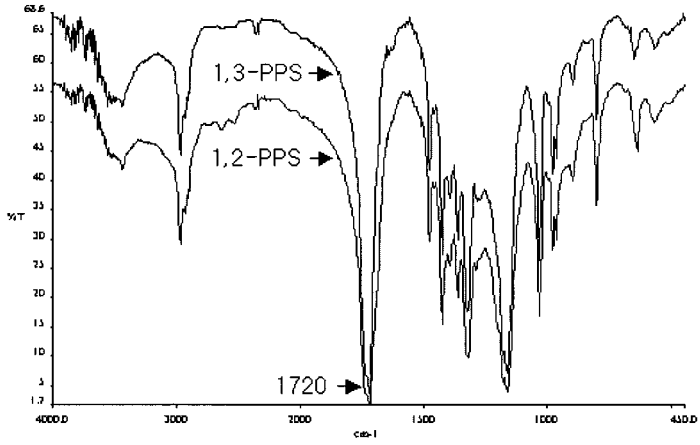

Figure 3. FT-IR spectra of poly(1,2-propanediol succinate) and poly(1,3-propanediol succinate).

evolved during catabolism. In this study, poly(1,2-propanediol succinate) and poly(1,3-propanediol succinate) were treated in an activated sludge under aerobic conditions, and the carbon dioxide that evolved was quantitatively determined as barium carbonate. The results are graphically represented in Figure 4. The evolution of carbon dioxide in the catabolism of poly(1,2-propanediol succiante) in the activated sludge was slower than in the case of poly(1,3-propanediol succinate). The difference can be explained by hydrophilicity and hydrophobicity. Poly(1,2-propanediol succinate) is more hydrophobic because of the pendant methyl group than poly(1,3-propanediol succinate). Microorganisms are more likely to interact with hydrophilic polymers. Therefore, poly(1,3-propanediol succinate), as compared with poly(1,2-propanediol succinate), has a higher affinity to microorganisms and it is more susceptible to biodegradation. As shown in Figure 4, the amount of carbon dioxide in the initial stage became negative; that is, the amount of carbon dioxide evolved in the run with a polymer sample was smaller than that in a control experiment without samples. The polymers are probably transformed to lower molecular weight carboxylic acids and/or alcohols before they are finally converted to carbon dioxide and water.

Biodegradation. A biodegradation test on polymer samples was carried out in an incubator controlled at 28°C. The biodegradation was followed by monitoring the

molecular weights of the remaining polymers by GPC, as shown in Figure 5 and Figure 6. As the GPC curves in Figure 5 indicate their characteristics clearly, the molecular weight of poly(1,2-propanediol succinate) decreased with increasing biodegradation time. Several

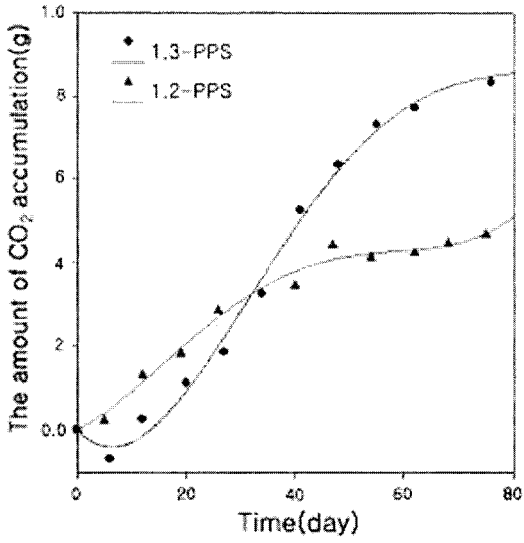

Figure 4. Evolution of carbon dioxide form of poly(1,2-propanediol succinate) poly(1,3-propanediol succinate).

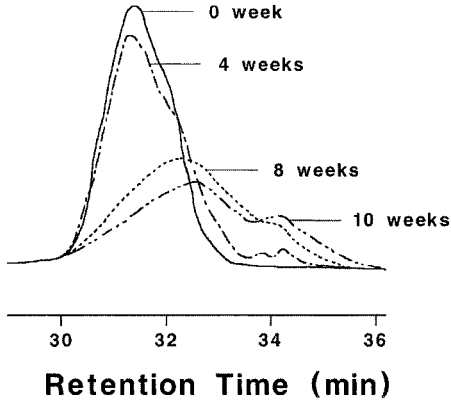

Figure 5. The GPC profiles of poly(1,2-propanediol succinate) before and after biodegradation by Aspergillus niger.

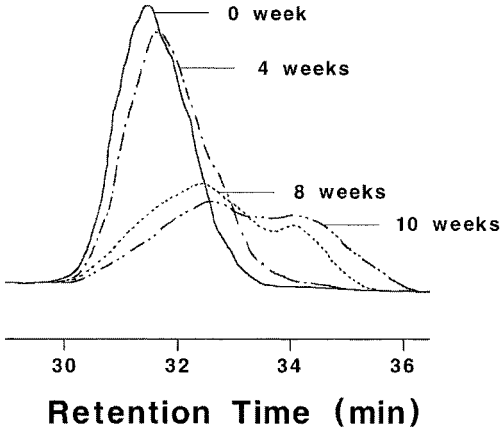

Figure 6. The GPC profiles of poly(1,3-propanediol succinate) before and after biodegradation by Aspergillus niger.

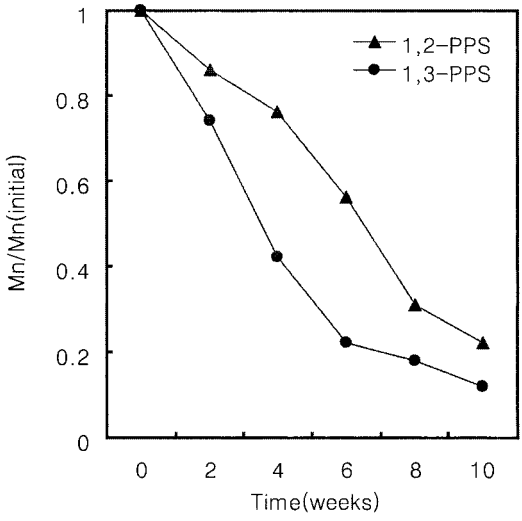

Figure 7. The changes of Mn/Mn(initial) of poly(1,2-propanediol succinate) and poly(1,3-propanediol succinate) with time in the biodegradation test of Aspergillus niger.

peaks, appearing in the low molecular weight region of the GPC curves, are ascribed to oligomeric compounds formed by biodegradation. The trend of GPC curves on poly(1,3-propanediol succinate) is similar to those of poly(1,2-propanediol succinate), as shown in Figure 6. But the areas of the low molecular weight region in the chromatogram of poly(1,3-propanediol succinate) increased faster than that of poly(1,2-propanediol succinate). Figure 7 compares the molecular weight decrease of poly(1,2-propanediol succinate) with that of poly(1,3-propanediol succinate). The biodegradation of poly(1,2-propanediol succinate) with the pendant hydrophobic methyl group was slower than that of poly(1,3-propanediol succinate).

Enthalpy changes of polymers during biodegradation. DSC thermograms of poly(1,2-propanediol succinate) before and after biodegradation by *Aspergillus niger* are shown in Figure 8. When poly(1,2-propanediol succinate) was heated from -20°C to 80°C at 10°C/min, T_m of pristine sample is shown at 47°C with broad melting endotherms. The broad melting endotherms observed in the sample are regarded as a superimposed peak of

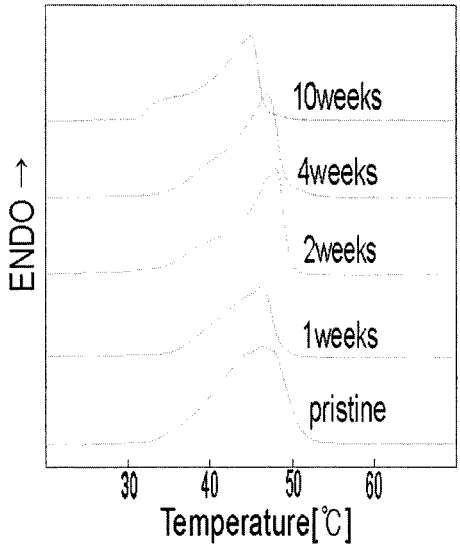

Figure 8. DSC thermograms of poly(1,2-propanediol succinate) before and after biodegradation by Aspergillus niger.

a few melting endotherms from the melt-recrystallization-remelt phenomena.[14,15] The melting temperature slightly increases with increasing degradation time but notably decreases after 10 weeks of biodegradation. The enthalpy changes($\triangle H_f$) of biodegradation samples decrease with increasing biodegradation time, as shown in Fig. 10. The increase of T_m and the decrease of enthalpy changes are due to the cleavage-induced crystallization resulting from the increase of chain mobility by chain scissions mainly in the amorphous region. The decrease of T_m for the sample in 10 week biodegradation is due to the degradation of the crystallization region as shown in the molecular weight decrease in the GPC data. The DSC thermograms of poly(1,3-propanediol succinate) are shown in Fig. 9. The melting temperatures and enthalpy changes of biodegradation samples decrease with increasing degradation time. These results are related to the fact that the chain scissions in the crystalline region of poly(1,3-propanediol succinate) proceed faster than those of poly(1,2-propanediol succinate) with the pendant hydrophobic methyl group.

Morphology change. The micrographs of polymer films degraded by micro-organisms are shown in Figure 11 and Figure 12, respectively. The spherulites of poly(1,2-propanediol succinate) and poly(1,3-propanediol succinate) are maintained after sterilization at 140°C for 4 hr as shown in Figure 11(a) and Figure 12(a). In the case of poly(1,2-propanediol succinate), the polymer film began to be covered by conidia and

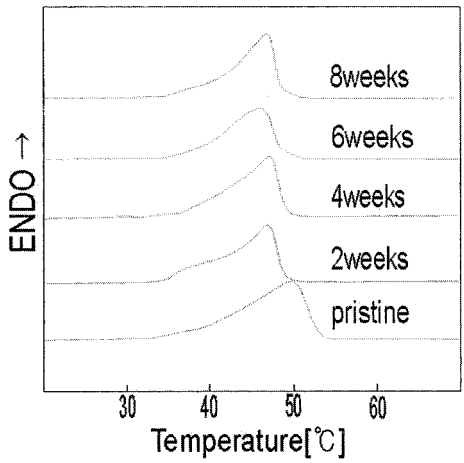

Figure 9. DSC thermograms of poly(1,3-propanediol succinate) before and after biodegradation by Aspergillus niger.

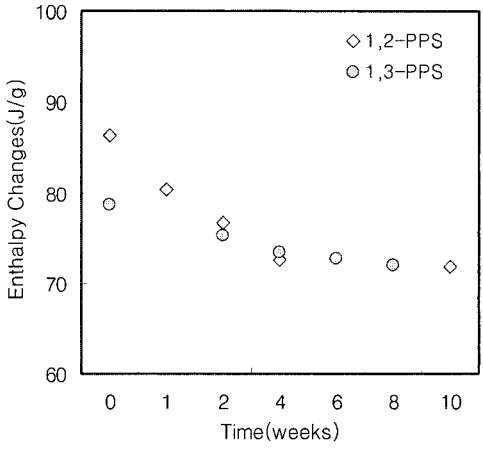

Figure 10. The enthalpy changes of poly(1,2-propanediol succinate) and poly(1,3-propanediol succinate) during biodegradation.

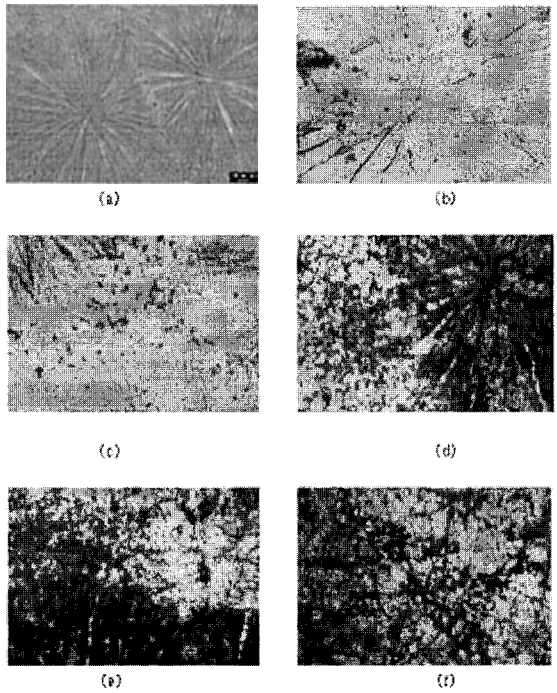

Figure 11. Biodegration of poly(1,2-propanediol succinate) by *Aspergillus niger*: (a) Before degradation, (b) 1 week, (C) 2 weeks, (d) 4 weeks, (e) 6 weeks, (f) 8 weeks.

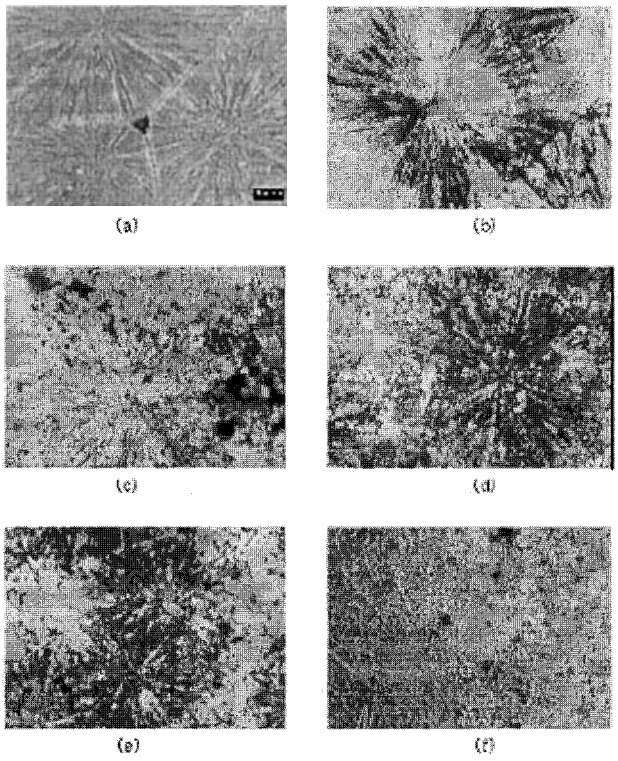

Figure 12. Biodegration of poly(1,3-propanediol succinate) by *Aspergillus niger*: (a) Before degradation, (b) 1 week, (C) 2 weeks, (d) 4 weeks, (e) 6 weeks, (f) 8 weeks.

mycelia after one week, as shown in Figure 11(b); the area covered by conidia and mycelia was wider after two weeks, as shown in Figure 11(c). After four weeks, as shown in Figure 11(d), the crystalline structure is maintained, but degradation proceeded in the amorphous area between spherulites. Some degraded areas are seen where no filaments are present after 6 weeks, as shown in Figure 11(e). The degradation seen along the mycelia is sufficient to cause the mycelia to sink into the surface of the film after 8 weeks, as shown in Figure 11(f). The biodegradation trends on poly(1,3-propanediol succinate), as shown in Figure 12(a – f), are similar to those on poly(1,2-propanediol succinate), as shown in Figure 11. However, the crystalline structure of poly(1,2-propanediol succinate) containing the pendant hydrophobic methyl group is maintained longer than that of poly(1,3-propanediol succinate) due to its hydrophobicity.

Conclusion

Biodegradable poly(1,2-propanediol succinate) and poly(1,3-propanediol succinate) were synthesized using Krebs cycle acid derivatives for biomodeling and biomedical applications. The weight average molecular weights of the synthesized samples were 8,900 in poly(1,2-propanediol succinate) and 8,600 in poly(1,3-propanediol succinate), respectively. The quantitative determination of carbon dioxide, generated during the treatment with the activated sludge, showed that poly(1,3-propanediol succinate) was biodegraded faster than poly(1,2-propanediol succinate) with the pendant hydrophobic methyl group. The biodegradability of *Aspergillus niger* to degrade the polymers was monitored using gel permeation chromatography. The biodegradation of poly(1,2-propanediol succinate) with the pendant hydrophobic methyl group was slower than that of poly(1,3-propanediol succinate) without the pendant hydrophobic methyl group. In the crystallinity changes of the polymers during biodegradation, the chain scissions in the crystalline region of poly(1,3-propanediol succinate) proceed faster than those of poly(1,2-propanediol succinate). Based upon the visual observation of their biodegradation, the crystalline structure of poly(1,2-propanediol succinate) retained its crystallinity longer than the similar structure in poly(1,3-propanediol succinate). These biodegradable polymers were degraded to the lower molecular weight components depending upon both their hydrophobicity and hydrophilicity of the chain structures.

Acknowledgement

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[1] T. H. Barrow, "Degradable implant materials", *Clin. Mater.*, **1986**, *1*, 233.
[2] B. Jeong, Y. H. Bae, D. S. Lee, and S. W. Kim, *Nature*, **1997**, *388*, 860.
[3] A. J. Domb, J. Kost, and D. M. Wiseman, *"Hand book of Biodegradable Polymer"*, Harwood Academic Publishers, The Netherlands, 1997.
[4] M. Chain and R. Langer (eds.), *"Biodegradable Polymers as Drug Delivery Systems"*, Marcel Dekker Inc., New York, 1990.
[5] D. Shum-Tim, U. Stock, J. Hrkach, T. Shinoka, J. Lien, M. A. Moses, A. Stamp, G. Taylor, A. M. Moran, W. Landis, R. Langer, J. P. Vacanti, and J. E. Mayer Jr., *Ann. Thorac. Surg.*, **1999**, *68*, 2298.
[6] H. Nishida and Y. Tokiwa, *J. Appl. Polym. Sci.*, **1992**, *46*, 1467.
[7] M. Okada, S. Ito, K. Aoi, and M. Atsumi, *J. Appl. Polym. Sci.*, **1994**, *51*, 1045
[8] D. K. Song, J. S. Lee, and Y. K. Sung, *Polymer(Korea)*, **1993**, *17*, 710.
[9] D. K. Song and Y. K. Sung, *J. Appl. Polym. Sci.*, **1995**, *56*, 1381.
[10] Y. K. Sung and S. J. Han, *Polymer(Korea)*, **1999**, *7*, 1.
[11] Y. K. Sung, M. S. Oh, D. K. Song, and S. W. Kim, *Biomaterials and Drug Delivery toward New Millenium*, K. D. Park, I. C. Kwon, N. Yui, S. Y. Jeong, and K. Park (Eds.), 2000, 669-685.
[12] Y. K. Sung and S. J. Han, *Polymer(Korea)*, **2001**, *25*, 108.
[13] American Standards for Testing and Materials, ASTM D G21-70, 1985; *Annual Book of Standards*, American Standards for Testing and Materials, Philadelphia, PA, 1985, 1052.
[14] M. E. Nichols and R. E. Robertson, *J. Polym. Sci. Polym. Phys.*, **1992**, *B(30)*, 755.
[15] J. S. Shin, E. S. Yoo, S. S. Im, and H. H. Song, *Polym(Korea)*, **2001**, *9*, 210.