Preparation of Porous Poly(D,L-lactide) and Poly(D,L-lactide-*co*-glycolide) Membranes by a Phase Inversion Process and Investigation of Their Morphological Changes as Cell Culture Scaffolds

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ABSTRACT: Porous membranes composed of the biodegradable polyesters poly(D,L-lactide) (PLA) and poly(D,L-lactide-*co*-glycolide) (PLGA) were prepared by a phase inversion process. The molecular weights of the polymers and the concentrations of the polymer solutions affected the pore size and structure of the PLA and PLGA membranes. The molecular weights and morphological changes of the membranes as a function of time were investigated under incubation at 37°C in a humidified 5% CO_2 atmosphere. The pores that formed in the membranes changed dramatically with increasing time under these conditions. From the thermal characterization of the polymers in their dry and wet states, we found that the glass-transition temperatures of PLA and PLGA affected morphological structure changes in the porous membranes. We also prepared a collagen-coated membrane to improve the interaction between the cell and the substrate, and we observed that the collagen coating enhanced the attachment and growth of Chinese hamster ovary cells on the substrate. Finally, we found that only PLA was a suitable material to prepare a porous membrane scaffold with the phase inversion process with PLA, and a collagen coating was necessary for cell culture on the membrane. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 92: 2082–2092, 2004

Key words: biodegradable; biocompatibility; relaxation

INTRODUCTION

Reconstruction of defective tissue with graft materials has been a widely used procedure. Recently, the engineering of new tissues by means of cell transplantation has emerged as a promising approach to the treatment of a great variety of clinical problems.[1–5] In particular, the reconstruction of tissue by the seeding of cultured cells in a synthetic biodegradable polymer scaffold is an attractive approach to the creation of devices for the restoration or modification of tissue or organ function.[1–5]

In many tissue engineering applications, porous scaffolds with an open-pore structure are often desirable because they maximize cell seeding, attachment, and growth; extracellular matrix (ECM) production; vascularization; and tissue ingrowth.[5–7]

In the field of polymer membrane science and technology, there are aspects of membrane structure, such as pore size and pore-size distribution, that can be controlled for each specific application. The development of the phase inversion technique preceded many systematic studies of the effects of the mechanism and relevant parameters of membrane formation.[8–14] Asymmetric membranes have been developed by the phase inversion method, which is a versatile technique that allows one to obtain all kinds of morphologies. In this process, a polymer solution is cast on a suitable support and then immersed in a coagulation bath containing a nonsolvent. The interchange of solvent and nonsolvent due to diffusion causes the casting solution to undergo a phase transition by which the membrane is formed.[8–14] The membrane structure ultimately obtained results from a combination of mass transfer and phase separation.

Synthetic biodegradable polymers have become very important as biomaterials for applications in tissue engineering and controlled drug delivery.[15–23] Among these materials, biodegradable aliphatic polyesters derived from lactic and glycolic acids have been widely used either as temporary scaffolds for cell transplantation to regenerate various tissues or as carriers for the delivery of bioactive molecules. Polyesters, such as poly(D,L-lactide-*co*-glycolide) (PLGA) and poly(D,L-lactide) (PLA), can be easily processed into the desired configuration, and their physical, chemi-

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cal, mechanical, and degradation properties can be engineered to fit a particular need. They have low immunogenicity and toxicity, and their excellent biocompatibility has also been demonstrated in many biological systems.[15–23]

Many researchers have investigated and documented the biodegradation and biocompatibility of PLA and PLGA. In addition, tissue reactions to these polyesters have also been studied. Various polymeric microspheres, microcapsules, nanoparticles, pellets, implants, and films have been fabricated from PLA and PLGA.[15–23] However, most of the research has focused on their degradation properties, and there have been only a few studies on the morphological changes of porous scaffolds.[20–23]

Therefore, in this study, we concentrated on the morphological changes in the porous structure of PLA and PLGA membranes. We used a phase inversion process to obtain the membranes, which were then tested as supports for the culture of Chinese hamster ovary (CHO) cells. The molecular weights and morphological changes of the membranes as a function of time under the incubation conditions were investigated by means of gel permeation chromatography (GPC) and scanning electron microscopy (SEM), respectively. In addition, the thermal properties of the membranes were investigated by differential scanning calorimetry (DSC).

We also investigated the influence of a collagen coating on cell attachment to these membranes. Collagens, a group of proteins with similar structural characteristics, are the most abundant proteins in the body. Collagen is the major component of bone, skin, ligaments, and tendons. Because collagen, particularly type I collagen, possesses unique physicochemical, mechanical, and biological properties that make it suitable for tissue and organ repair, this protein has been extensively researched in past decades as a biomaterial for medical implant development.[24–28] We performed water-contact-angle (θ) measurements before and after the collagen coating of the substrates, and we investigated the attachment and growth of CHO cells cultured on the membrane.

EXPERIMENTAL

Materials

All of the polymers used in this study were purchased from Sigma-Aldrich (St. Louis, MO). The weight-average molecular weights (M_w) and number-average molecular weights (M_n) of the copolymers used were determined by GPC and are listed in Table I. Dimethylformamide (DMF) was obtained from Wako Pure Chemical Industries (Osaka, Japan). Collagen I (cell matrix type I-P, 3.0 mg/mL, pH 3.0) was obtained from Nitta Gelatin (Osaka, Japan).

TABLE I
Molecular Weights and Molecular Weight Distributions of PLA and PLGA Measured by GPC

No.	Polymer	LA/GA ratio	M_w^a	M_w^b	M_w/M_n
1	PLGA 50:50	50:50	40,000–75,000	44,700	1.78
2	PLGA 65:35	65:35	40,000–75,000	50,600	1.96
3	PLGA 75:25	75:25	90,000–126,000	90,400	2.04
4	PLGA 85:15	85:15	90,000–126,000	88,400	1.91
5	PLA	100:0	90,000–120,000	89,500	1.89

[a] Molecular weight by supplier (Sigma-Aldrich Japan).
[b] Determined by our GPC measurement.

The following cells and materials were used in the cell culture studies. CHO cells were purchased from the American Type Culture Collection (ATCC CHO-K1, Manassas, VA). Minimum essential medium–alpha modification (α-MEM; with L-glutamine, without ribonucleosides), Dulbecco's phosphate buffered saline, fetal bovine serum (FBS; Australian origin), and penicillin–streptomycin solution were obtained from Sigma-Aldrich. Trypsin–ethylenediaminetetraacetic acid (EDTA; 10×; 0.5% trypsin and 5.3 m*M* EDTA · 4Na) and trypan blue were purchased from GIBCO BRL (Grand Island, NY) and Wako Pure Chemical Industries, respectively. Ultrapure water was obtained by means of a Milli-Q Plus System (Waters-Millipore, Billerica, MA). All of the other chemicals were reagent grade and were used without further purification.

Preparation of the porous PLA and PLGA membranes by a phase inversion process

Porous PLA and PLGA membranes were prepared by a phase inversion technique. The basic procedure for the technique consisted of two steps. First, the polymer solution was cast over a suitable substrate to form a thin polymer film. Then, the film was immersed in a coagulation bath, where the solvent was replaced by the coagulant and the polymer was precipitated.

The PLA and PLGA copolymers were dissolved separately in DMF to form a 12–25 wt % polymer solution. The solution was cast on a glass plate in a uniform thickness of about 250 μm. The glass plate was immersed immediately in a coagulation bath in which the coagulant was water, and the temperature was maintained at 25°C. After 6 h, the resulting membrane was peeled from the glass plate and dried in air for 12 h and *in vacuo* for 24 h at room temperature.

Preparation of the collagen-coated PLA and PLGA membranes

Porous PLA and PLGA membranes were coated with type I collagen. A type I cell-matrix-type collagen solution (pH 3.0, concentration $\approx$ 0.3%) was diluted

with a *tris*-HCl buffer solution of pH 7.5. The collagen solution at a concentration of 0.075 mg/mL was adsorbed onto the PLA and PLGA membranes, and the membranes were incubated for 20 min at 25°C.

GPC measurements of PLA and PLGA

The molecular weight distributions and the average molecular weights of PLA and PLGA were determined by a GPC instrument (model 576 HPLC pump, GL Science, Tokyo, Japan) equipped with a refractive index detector (Shodex RI, SE-51, Showa Denko, Tokyo, Japan). The samples were dissolved in tetrahydrofuran and eluted through a Shodex packed column (model GPC KF-805, Showa Denko) at a flow rate of 1.0 mL/min. Before the measurements were taken, a calibration curve was prepared with polystyrene standards (molecular weights = 1.31×10^4, 7.22×10^4, 2.15×10^5, 6.29×10^5, and 1.29×10^6; Shodex Standard SM-105, Showa Denko).

DSC measurements of the PLA and PLGA membranes

DSC was performed on a DSC220 (Seiko Instruments, Inc., Chiba, Japan). The calorimeter was calibrated with an indium standard sample. The PLA and PLGA membrane samples were sealed in aluminum pans and heated twice under a nitrogen atmosphere. The thermograms were recorded at a heating and cooling rate of 10°C/min. The second run was used for the calculation of the glass-transition temperatures (T_g's). The PLA and PLGA membrane samples were vacuum-dried for at least 24 h at 25°C before they were tested. For measurements of wet samples, hydrated membrane samples were used.

θ measurement

Four samples, collagen-coated PLA and PLGA membranes and uncoated PLA and PLGA membranes, were cut to 1.5 × 1.5 cm for the measurement of θ's. The angles were determined by the sessile drop method at 25°C with a FACE contact-angle meter (type CA-DT-A, Kyowa Interface Science, Saitama, Japan). Each PLA and PLGA membrane sample was measured five times, and the average angle and the standard deviation were calculated.

Observation of the morphological structures of the PLA and PLGA membranes

The morphological structures of the porous PLA and PLGA membranes prepared by the phase inversion method were examined with a scanning electron microscope (DS-720, TOPCON, Tokyo, Japan). The samples were coated with gold by means of a plasma multicoater (PMC-5000, Meiwa, Tokyo, Japan). The current was maintained at 10 mA for a coating time of 3.5 min.

Cell culture experiments

To investigate the influence of the collagen coating on cell attachment, CHO cells were cultured on a tissue culture polystyrene (TCPS) dish and on the dense PLA membrane before and after collagen coating. CHO-K1 cells from the American Type Culture Collection (ATCC CHO-K1) were cultured in a α-MEM with 10% FBS in an incubator at 37°C in a humidified 5% CO_2–air atmosphere. After a monolayer confluent propagation, a 0.2% trypsin solution was added for the detachment of the cells from the TCPS dish. The cell suspension was adjusted to a concentration of 7.5×10^5 cells/mL. The cell suspension was seeded onto the 1.5 × 1.5-cm TCPS and prewetted PLA membranes at a density of 7.0×10^4 cells/cm^2. All of the collagen-coated and uncoated PLA membranes were sterilized by ultraviolet irradiation for 20 min before cell seeding. The cells were allowed to attach to the substrates in a humidified incubator (37°C and 5% CO_2) for 24 h. The attachment of CHO cells cultured on these collagen-coated substrates was compared with the attachment of cells cultured on the uncoated membranes as a function of time. CHO cells cultured on the TCPS membrane and the dense PLA membrane were observed with a phase inversion microscope (IX70, Olympus Optical, Japan) and a zoom stereomicroscope (SZH-12, Olympus Optical, Tokyo, Japan), respectively.

Furthermore, the attachment and growth of CHO cells on the collagen-coated membranes were investigated under the cell culture conditions described previously. After 7 days of incubation (α-MEM containing 10% v/v FBS under a 5% CO_2 atmosphere at 37°C), the CHO cells on the collagen-coated porous PLA membrane were also observed with the zoom stereomicroscope.

RESULTS AND DISCUSSION

Porous PLA and PLGA membranes prepared by the phase inversion method

In the phase inversion process, a solution consisting of a polymer and a solvent is cast as a thin film upon a support and then immersed into a nonsolvent coagulation bath. The interchange of solvent and nonsolvent due to diffusion causes the casting solution to go through a phase transition by which the membrane is formed.[8–14] In other words, the solvent diffuses into the coagulation bath, and the nonsolvent diffuses into the cast film. After a given period of time, the ex-

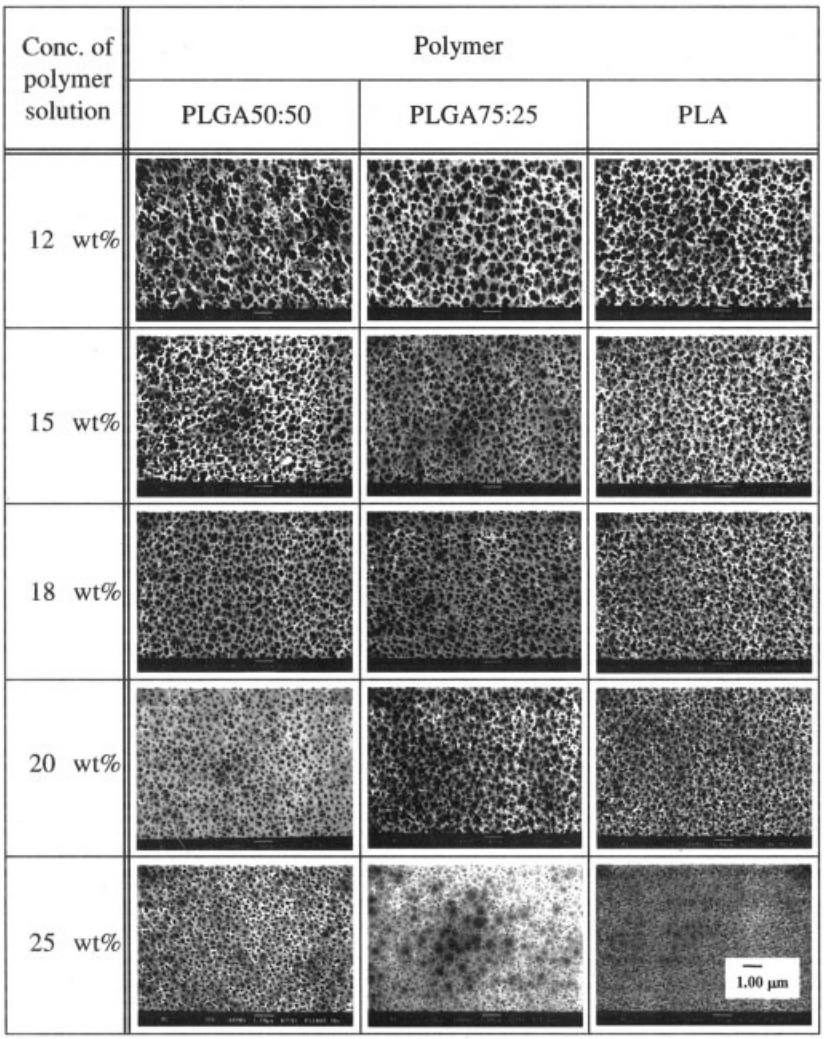

Figure 1 Effects of polymer concentration and composition on the morphological structures of porous PLA and PLGA membranes prepared by the phase inversion method.

change of solvent and nonsolvent proceeds so far that the solution becomes thermodynamically unstable, and demixing takes place. Finally, a solid polymeric film with an asymmetric structure is obtained. Therefore, the thermodynamic properties of a polymer–solvent–nonsolvent system are strongly influenced by the solvent quality and the solvent–nonsolvent interactions. One of the main variables in the phase inversion process is the choice of the solvent–nonsolvent system. For the preparation of a membrane from a polymer by phase inversion, the polymer must be soluble only into the solvent, and the solvent and nonsolvent must be completely miscible. Therefore, in this study, we used DMF as the solvent and water as the nonsolvent.

Figure 1 shows the porous surface structure of PLA and PLGA membranes prepared by the phase inversion method. From these SEM images, we observed that the porous structures of the membranes differed considerably, depending on the concentration of the polymer solution used in the phase inversion process. As shown in Figure 1, which we obtained by varying the initial polymer concentration in the casting solution from 12 to 25 wt %, the porosity and pore size of the membranes gradually decreased as the concentration of the polymer solution increased. Increasing the initial polymer concentration in the casting solution led to a much higher polymer concentration at the interface. This result implies that the volume fraction of the polymer increased, and consequently, a lower porosity was obtained.

In contrast, the composition ratio of lactide and glycolide in the copolymer did not significantly influence the porous structure of the PLA and PLGA membranes. Therefore, the structure of membranes prepared by the phase inversion method, especially pore size and pore distribution, could be controlled for each specific application by the choice of the polymer, solvent, and nonsolvent and by the preparation parameters, such as polymer concentration.

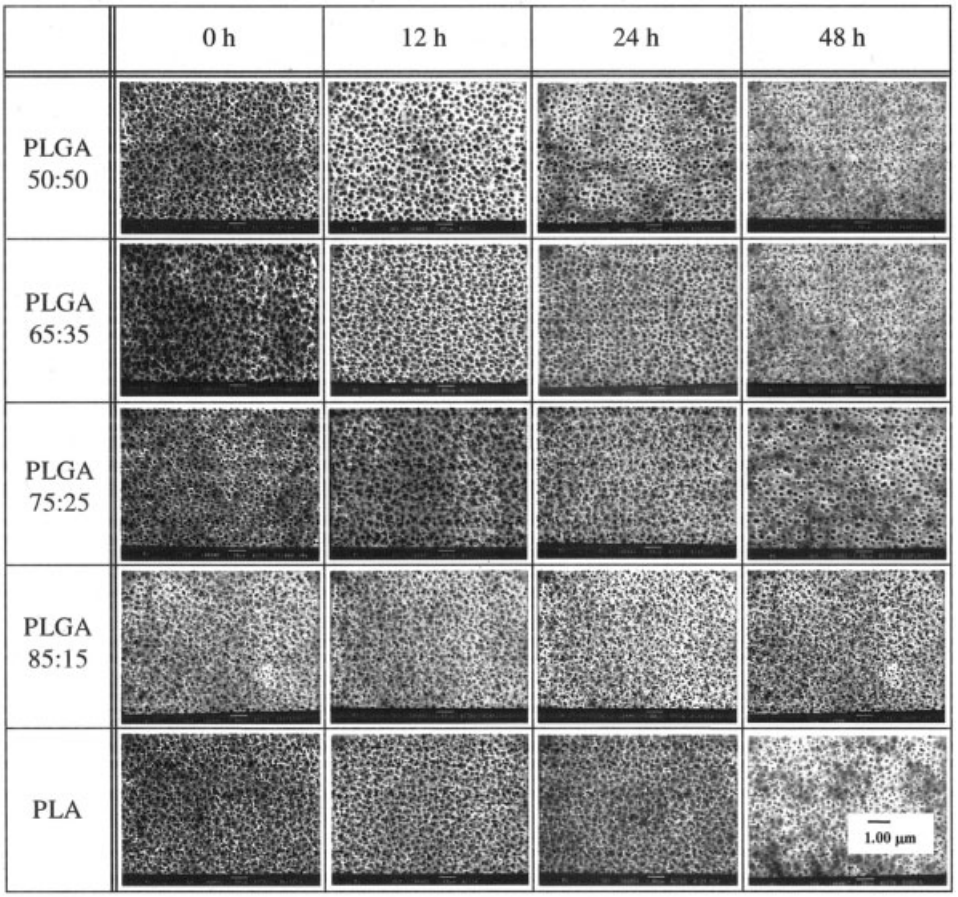

Figure 2 Scanning electron micrographs of the changes in the surface structures of the porous PLA and PLGA membranes incubated at 40% humidity in a 5% CO_2 atmosphere at 37°C as a function of time.

Morphological analysis of PLA and PLGA

For application of the membranes as scaffolds in tissue engineering, a porous membrane structure is essential for cellular adaptation and sufficient nutrient permeation.[5–7] Therefore, we used SEM to investigate the morphological changes in the porous PLA and PLGA membranes as a function of time. Figure 2 shows the surface structure of the porous membranes under incubation conditions of 5% CO_2 and 40% humidity at 37°C. As shown in Figure 2, the porosity and pore size of the membranes decreased somewhat with time.

In addition, we observed the surface structure of the PLA and PLGA membranes under 5% CO_2 at 100% humidity and 37°C. The morphological changes in the porous membranes occurred more rapidly at 100% humidity than at 40% humidity (Fig. 3). As the composition ratio of D,L-lactide to glycolide in the copolymer decreased, the structural changes became severe. In particular, the change in the structure of the PLGA membrane prepared with PLGA 50:50 was much faster than the changes in the other membranes.

However, we observed that the porous structure of the PLA and PLGA membranes did not change under dry conditions at room temperature. These results indicate that temperature and humidity affected the porous structure of these membranes.

Changes in the molecular weights of PLA and PLGA

To determine the molecular weights of the PLA and PLGA polymers during the change in the membranes' morphological structure, we determined the polymers' molecular weights by GPC under the conditions used for the morphological analysis mentioned previously.

Figures 4 and 5 show the molecular weights of the PLA and PLGA membranes as a function of time at 40 and 100% humidity at 37°C, respectively.

Both *in vitro* and *in vivo,* the PLGA copolymer degrades in an aqueous environment (by hydrolytic degradation or biodegradation) through cleavage of its backbone ester linkages.[20–23] The PLGA polymer biodegrades into lactic and glycolic acids. Lactic acid enters the tricarboxylic acid cycle and is metabolized and subsequently eliminated from the body as carbon dioxide and water. Glycolic acid is either excreted by

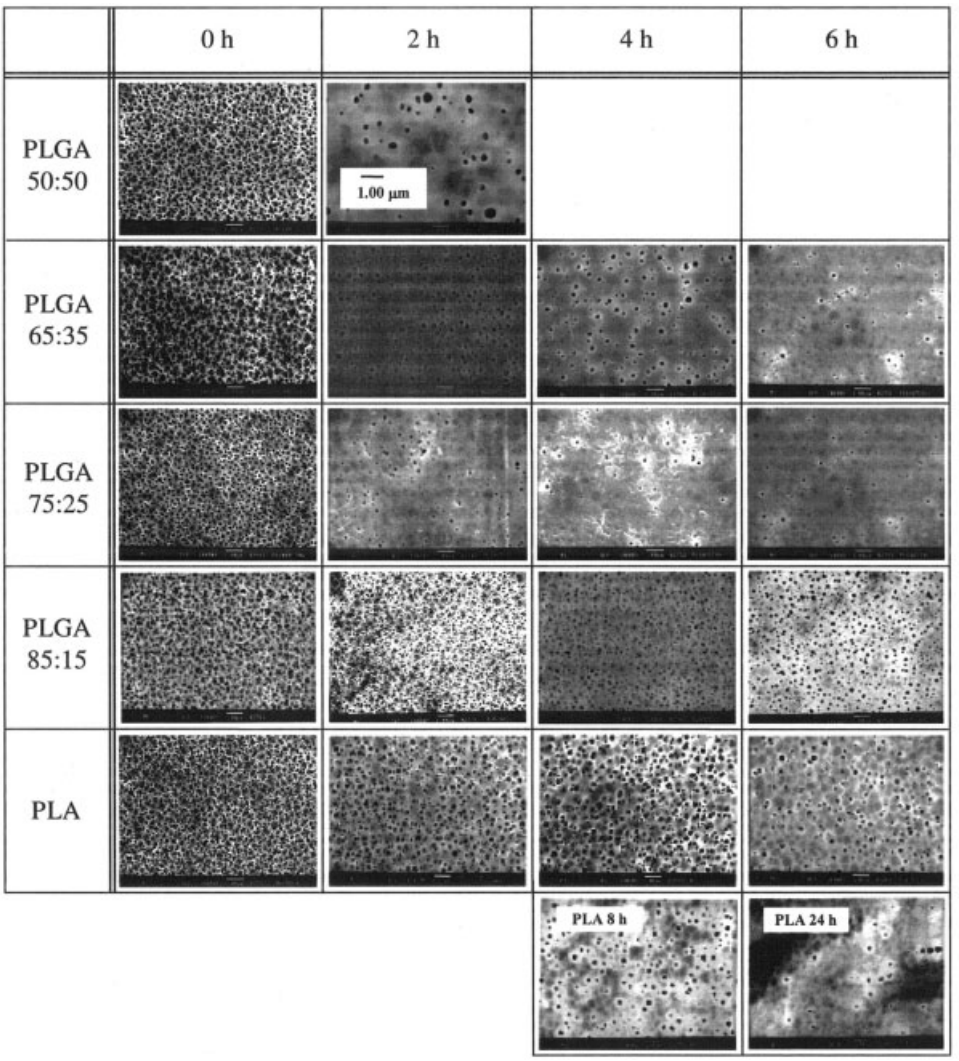

Figure 3 Morphological changes in the surface structures of the porous PLA and PLGA membranes incubated at 100% humidity in a 5% CO_2 atmosphere at 37°C as a function of time.

the kidneys or eliminated as carbon dioxide and water via the tricarboxylic acid cycle. The biodegradation rate of the PLGA copolymers depends on the molar ratio of the lactic and glycolic acids in the polymer chain and on the polymers' molecular weight, degree of crystallinity, and T_g.[20–23]

In the GPC measurements, as expected, the PLA and PLGA membranes showed no change in molecular weight within 48 h at either 40 or 100% humidity. Therefore, we confirmed that the molecular weights of PLA and PLGA did not change during the morphological structure change of the membranes and did not influence the changes in the porous structure of the membranes.

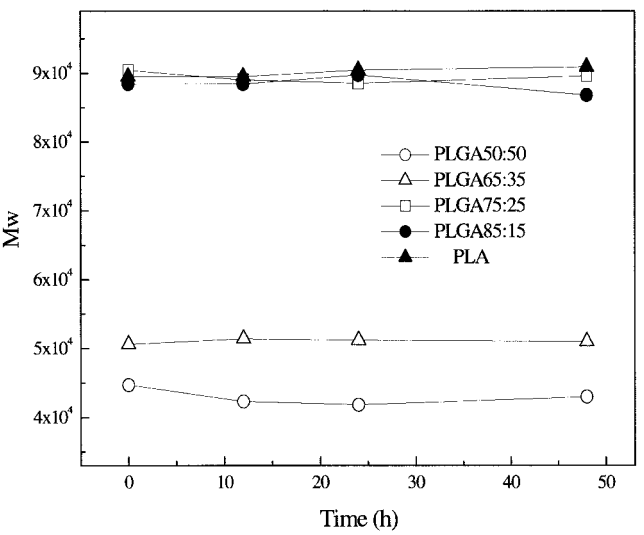

Figure 4 M_w's of the porous PLA and PLGA membranes incubated at 40% humidity in a 5% CO_2 atmosphere at 37°C as a function of time.

T_g values of the PLA and PLGA membranes

Glass-transition analysis was also carried out by means of DSC. Figure 6 shows the DSC thermograms of the porous PLA and PLGA.

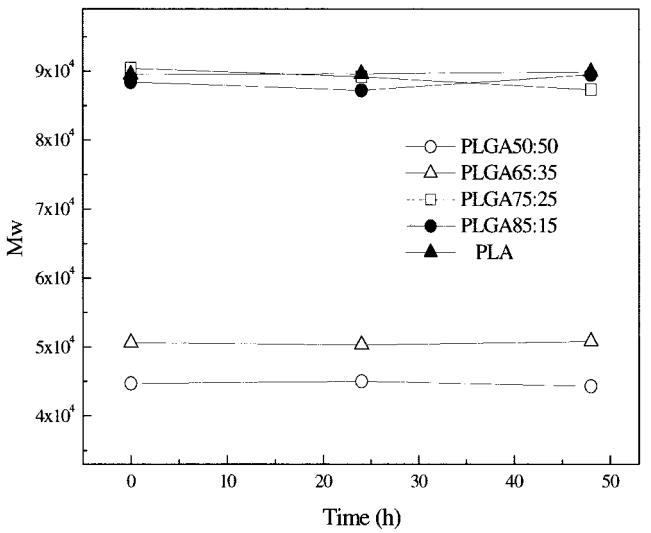

Figure 5 M_w's of the porous PLA and PLGA membranes in water incubated at 100% humidity in a 5% CO_2 atmosphere at 37°C as a function of time.

The T_g values for the membrane samples are summarized in Table II. As shown in Table II and Figure 6, the T_g values of the PLGA membranes decreased as the copolymers' lactide content and molecular weight decreased. The dried PLA membrane prepared with the inoptically inactive racemic form of the polymeric PLA homopolymer [P(D,L)LA] showed a T_g of around 49°C, and the T_g of the dried PLGA 50:50 membrane,

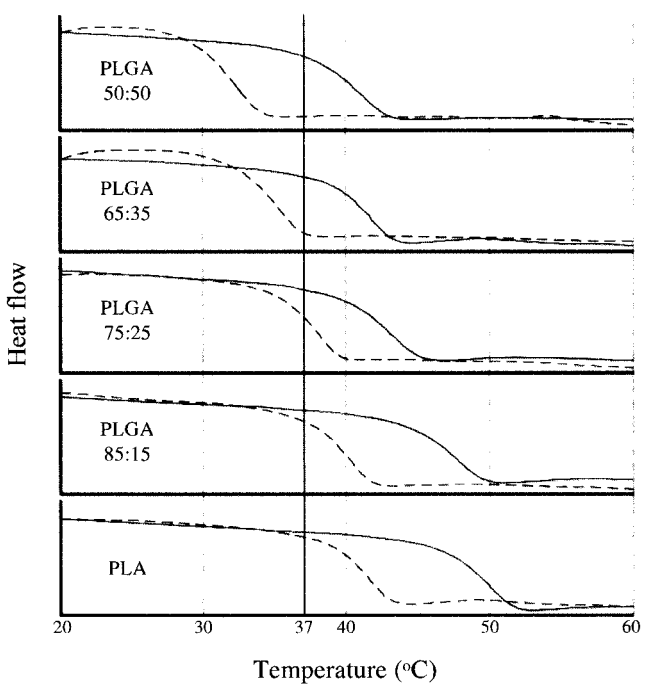

Figure 6 Thermograms of PLA and PLGA membranes determined by DSC under (—) dry and (–) wet conditions.

TABLE II
T_g Values of PLA and PLGA Membranes Determined by DSC

No.	Polymer	T_g (°C) Dry state	T_g (°C) Wet state
1	PLGA 50:50	40.3	31.2
2	PLGA 65:35	42.3	34.4
3	PLGA 75:25	45.2	37.3
4	PLGA 85:15	46.6	39.6
5	PLA	49.0	41.2

which had a lower D,L-lactide content than the other PLGA membranes, was 40.3°C.

Polymeric PLA can exist in an optically active stereoregular form [P(L)LA] and as P(D,L)LA. P(L)LA is semicrystalline because of the high regularity of its polymer chain, whereas P(D,L)LA is an amorphous polymer because of irregularities in its polymer chain structure.[22,23,29] Polyglycolide (PGA) is highly crystalline because it lacks the methyl side groups of PLA. Hence, the crystallinity of the PLGA copolymer depends on the type and the molar ratio of the individual monomer components (lactide and glycolide) in the copolymer chain. PLGAs prepared from P(L)LA and PGA are crystalline, whereas those prepared from P(D,L)LA and PGA are amorphous. In particular, it is known that the crystallinity of PLGA polymers containing a 50:50 ratio of lactide and glycolide is significantly lower than that of polymers containing a higher proportion of either of the two monomers.[22,23,29] Therefore, as shown in Figure 6 and Table II, the dependence of T_g on the lactide and glycolide content in the PLGA copolymer was attributed to the increase in amorphous regions in the PLGA copolymer.

Wetted membrane samples showed lower T_g values than the dry samples. The T_g values of all the wetted PLGA membranes, except PLGA 85:15, were below the physiological temperature of 37°C. In the hydrated PLA and PLGA membranes, molecules of water, which can act as a plasticizer, diffused between the polymer chains. This effect could have been the cause of the decreasing T_g values for the PLA and PLGA membranes.

The DSC thermograms indicated that the morphological changes in the porous structure of the membranes under humid incubation conditions at 37°C may have been influenced by the change in the T_g values of the PLA and PLGA membranes. As mentioned previously, the morphological structure of the membranes changed considerably with temperature and humidity and accelerated at high temperatures and humidities. The results from the morphological analysis were in accord with the results from the DSC thermograms. Therefore, from the results, we con-

TABLE III
Average Values of θ for PLA and PLGA Membranes with and Without Type I Collagen coatings

No.	Sample	Collagen coating[a]	θ[b]
1	PLA	Uncoated	71.9 ± 1.6
2	PLGA 50:50	Uncoated	82.7 ± 2.9
3	C-PLA	Coated	69.3 ± 2.1
4	C-PLGA 50:50	Coated	65.2 ± 0.9

[a] Type I collagen solution (0.075 mg/mL) was adsorbed onto PLA and PLGA membranes, and the membranes were incubated for 20 min at 25°C.
[b] Mean ± standard deviation (n = 5 for each sample).

cluded that the T_g values for PLA and PLGA played an important role in the formation of the porous structure of the PLA and PLGA membranes prepared by the phase inversion process.

Because the PLA membrane showed less morphological change in its porous structure than the PLGA membrane, we investigated cell adhesion and growth on with the PLA membrane as a substrate.

Surface characteristics of collagen-coated and uncoated PLA and PLGA membranes

The surface chemistry of a material can mediate the cellular response to the material and, in turn, affect cell function on the surface, cell adhesion, proliferation, and migration.[30–32] In particular, the wettability of a polymer scaffold is considered very important for homogeneous and sufficient cell seeding in three dimensions.

Although the polyesters PLA and PLGA show good biocompatibility and degradability, it is difficult use them to deliver a cell suspension in a manner that distributes transplanted cells uniformly throughout the scaffold because these polyesters are relatively hydrophobic. Hydrophobic polymers are known to be unfavorable for cell attachment unless they are modified so that they possess a hydrophilic surface with a higher surface energy and a correspondingly low θ.[33,34]

Therefore, in this study, we coated PLA and PLGA membranes with collagen, which is hydrophilic, and we determined the θ's for the membranes. Table III shows the θ data for each surface that were obtained by means of the sessile drop method. The hydrophobic characteristics of the untreated PLA and PLGA surfaces showed relatively higher θ's in comparison with the collagen-coated PLA and PLGA membranes, as shown in Table III. This difference was probably due to the presence of a hydrated layer that persisted in the interface between the collagen-coated surface and water.

Type I collagen is a major biological macromolecule of the ECM in tissues and, because of its biological properties, is an attractive molecule for the manufacture of biomaterials.[24–28] Therefore, a collagen coating on the PLA and PLGA membranes, which are bioinert and have no biological functions, could provide a favorable biological environment for interactions with substrates and cells.

From phase contrast micrographs of CHO cells cultured on an untreated TCPS dish and a collagen-coated TCPS dish as a function of time (Fig. 7), we observed that the type I collagen coating on the TCPS surface significantly enhanced initial cell attachment and increased cell adhesion as the culture time increased.

In addition, we investigated the effect of the collagen coating on the morphological characterization of CHO cells cultured on the dense PLA membrane. Figure 8 shows the CHO cells cultured on untreated and collagen-coated dense PLA membranes after 24 h of culturing as observed by zoom stereomicroscopy. As shown in Figure 8, CHO attachment on the collagen-coated PLA membrane was also significantly promoted relative to attachment on the untreated PLA membrane. These results suggest that the type I collagen coating allowed the CHO cells to interact with the collagen surfaces and to avoid direct interaction with PLA surfaces and that the coating then played a useful role in the support of the initial attachment and growth of the CHO cells. That is, the result probably arose because the collagen coating could eliminate the adsorption of extracellular matrices onto the surface of the material and, thus, could make the cells bind to the surface easily, quickly, and totally. This improved adsorption increased the total number of bonds formed between the cell and the coated surface.

Morphological characterization of CHO cells cultured on the porous PLA membrane

A three-dimensional, porous, and mechanically stable structure is desirable for cell growth and for the retention of differentiated cell function. A uniformly distributed pore structure is especially important for the formation of an organized network of tissue constituents because such a structure can provide adequate space for cell seeding, growth, and ECM production.[5–7]

Therefore, cellular growth was also tested on the porous PLA membrane prepared by the phase inversion method. Figure 9 shows CHO cells cultured on the collagen-coated porous PLA membranes after 7 days of culture.

Cell adhesion to a substrate can be regarded as a two-step process.[30–32] The first step is controlled by complex combinations of physicochemical interactions, including hydrophobic, Coulombic, and van der

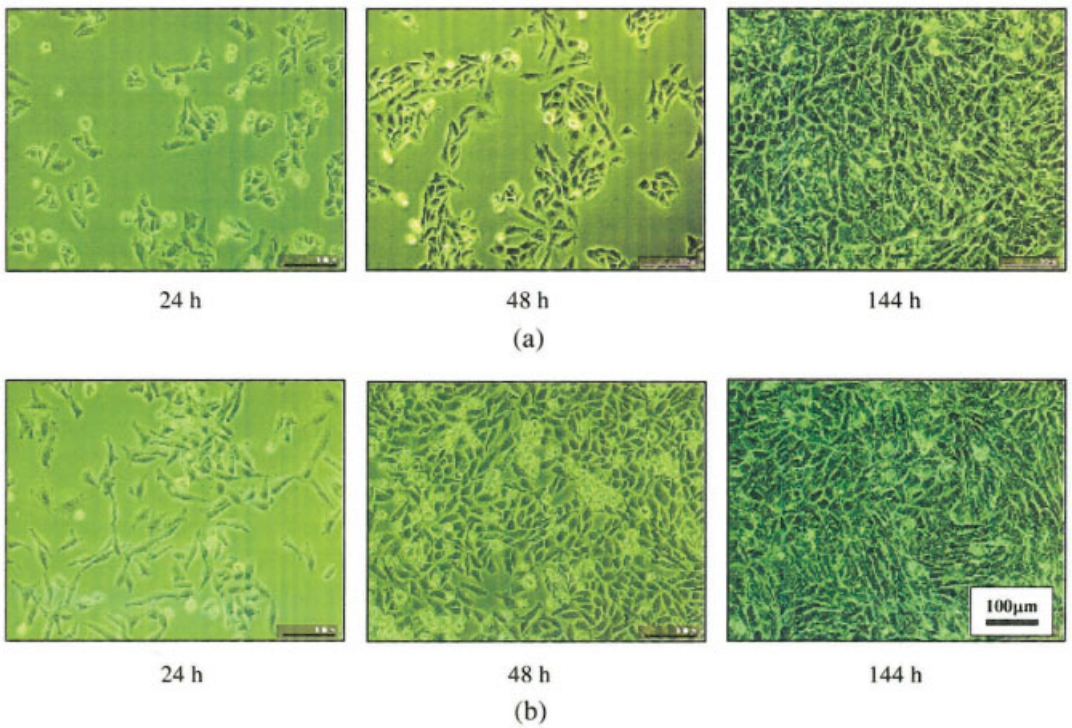

Figure 7 Phase contrast micrographs of CHO cells cultured on the (a) untreated TCPS dish and (b) collagen-coated TCPS dish as a function of time.

Waals forces between the cell and the surface. The process might be regarded as passive adhesion. The second step can be regarded as active adhesion because of the participation of cellular metabolic processes, including the construction of focal adhesions and cytoskeletal recognition.[30–32]

As seen in the zoom stereomicroscopic image in Figure 9, both elongated CHO cells and round CHO cells were observed. Although the PLA membranes underwent some morphological changes in their porous structure when incubated under high humidity at 37°C, these conditions did not significantly affect the adhesion and growth of the CHO cells. These results indicate that the collagen coating may have enhanced both the passive adhesion that occurred during first step (hydrophilicity) and the active adhesion that occurred during the second step (molecules of the ECM). Then, the porous structure formed by the phase inversion process could have reserved sufficient free space for the formation of new tissue via cell proliferation and ECM secretion.

CONCLUSIONS

Biodegradable porous PLA and PLGA membranes were prepared by means of a phase inversion method. The porosity and pore sizes of these membranes were mainly influenced by the molecular weight of the

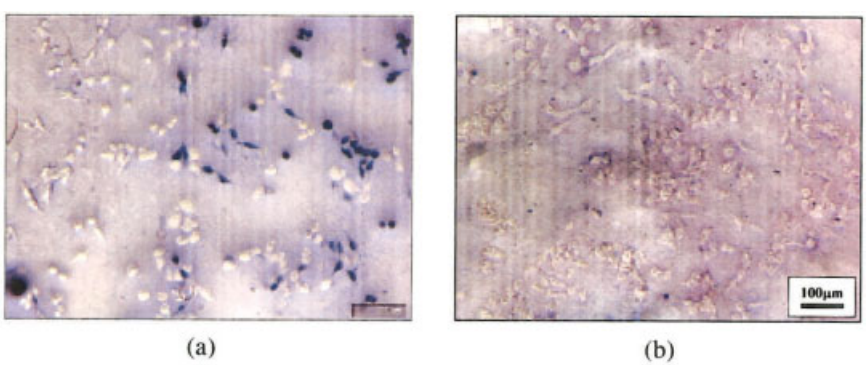

Figure 8 CHO cells cultured on the (a) untreated dense PLA membrane and (b) collagen-coated dense PLA membrane after 24 h of culturing.

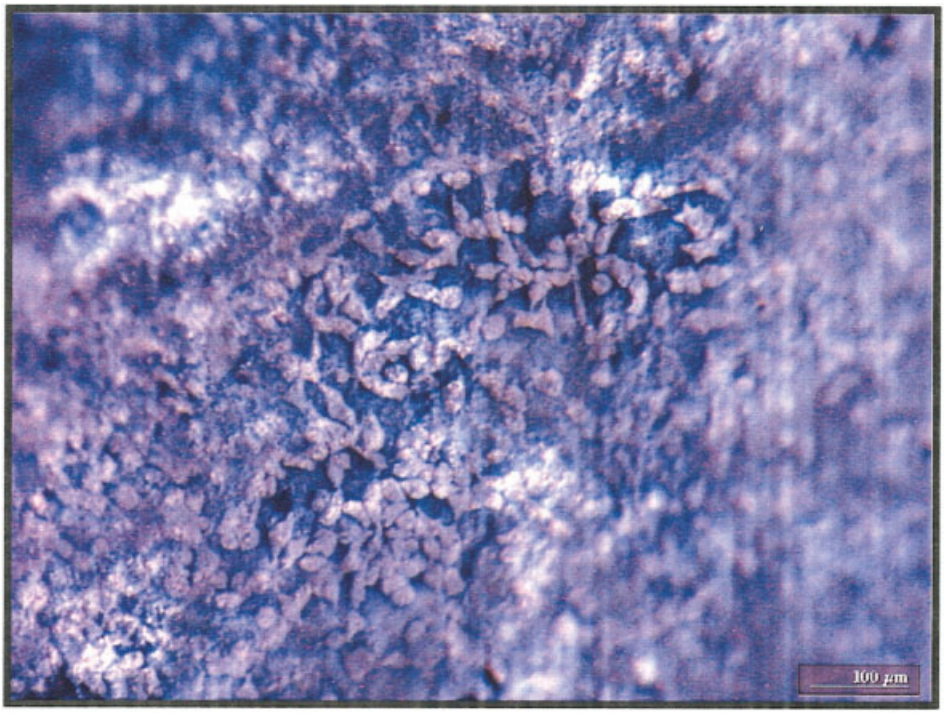

Figure 9 CHO cells cultured on the collagen-coated porous PLA membrane after 7 days of culturing.

polymers and by the concentration of polymer solutions; the composition ratio of lactide and glycolide in the copolymers did not significantly affect the porous structure. Under high humidity at 37°C, the pore morphology of the membranes was strongly influenced by temperature and humidity, and this was attributed to changes in the T_g values of the membranes. In addition, porous PLA and PLGA membranes coated with type I collagen were prepared to enhance the interaction between the cells and the substrate. From the θ's and the culture experiments on CHO cells, we found that the collagen coating improved the CHO cell attachment by increasing the hydrophilicity of the polyester substrates, which were relatively hydrophobic, and by introducing biological properties. In addition, we observed CHO cells with elongated and round shapes on the collagen-coated porous PLA membrane after 7 days of culturing.

Finally, we found that only PLA was suitable as a material to prepare a porous membrane scaffold with the phase inversion process with PLA, and the collagen coating was necessary for cell culture on the membrane.

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