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Degradation behavior of hydrophilized PLGA scaffolds prepared by melt-molding particulate-leaching method: Comparison with control hydrophobic one

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Porous PLGA/PVA scaffolds as hydrophilized PLGA scaffolds for tissue engineering applications were fabricated by a novel melt-molding particulate leaching method (non-solvent method). The prepared scaffolds exhibited highly porous and open-cellular pore structures with almost same surface and interior porosities (pore size, 200–300 μm; porosity, about 90%). The *in vitro* degradation behavior of the PLGA and PLGA/PVA scaffolds was compared at 37°C in PBS (pH 7.4) with and without the solution change everyday to see the effect of solution pH as well as scaffold hydrophilicity on the degradation behavior. The changes in dimension, molecular weight, mechanical properties (maximum load and modulus), and morphology of the scaffolds were examined with degradation time. The degradation behavior of the PLGA and PLGA/PVA scaffolds was further investigated *in vivo* using a rat model (subcutaneously implantation). It was observed that both PLGA and PLGA/PVA scaffolds in decreasing pH condition (PBS no change) showed faster degradation than those in constant pH condition (PBS change everyday), owing to the enhanced intramolecular depolymerization by the increment of chain hydrophilicity caused by carboxylate groups as well as the autocatalysis of carboxylic acids accumulated in the solution by the cleavage of PLGA backbone ester bonds. The scaffolds *in vivo* condition also showed faster degradation than those *in vitro*, probably due to the aid of foreign body giant cells or enzymes. The PLGA/PVA scaffold showed slightly faster degradation than the PLGA scaffold for both *in vitro* and *in vivo* conditions.

Introduction

Recently biodegradable polymers have been used to fabricate porous scaffolds for three-dimensional (3-D) cell or tissue culture to regenerate tissue-based artificial organs. Poly(DL-lactic-co-glycolic acid) (PLGA) is one of the most widely used biodegradable polymers since it is biocompatible and its degradation rate can be easily controlled by varying the copolymer ratio of lactic to glycolic acid [1]. There have been several methods to fabricate porous biodegradable polymer scaffolds, including solvent casting/particulate leaching [2, 3], phase separation [4–6], emulsion freeze-drying [7], gas foaming [8, 9], gel casting [10], fiber bonding [11] and 3-D printing [12]. These scaffold fabrication methods typically use organic solvents, which may be harmful to cells, protein growth factors or nearby tissues when the residue remains in the scaffolds [13]. Among the scaffold fabrication methods, the solvent casting/particulate leaching method has been extensively utilized for the fabrication of porous cell scaffolds, however the scaffolds prepared by this method often exhibited a dense surface skin layer which is usually formed during the solvent evaporation step. The skin layer causes negative effects for *in vitro* cell seed-

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ing and culture or *in vivo* tissue regeneration into the scaffolds.

Recently, we fabricated porous PLGA scaffolds by a melt-molding particulate-leaching method designed by our laboratory [14]. The melt-molding particulate-leaching method does not involve any solvents during the scaffold fabrication process and the prepared scaffolds by this method show uniform 3-D porosity (almost same surface and interior porosities). The PLGA/polyvinyl alcohol (PVA) blend scaffolds were also fabricated to improve hydrophilicity and cell compatibility of the scaffolds. It was observed that the PLGA/PVA scaffolds with PVA compositions more than 5 wt% are easily wetted in cell culture medium [14]. As *in vitro* cell compatibility of the control hydrophobic PLGA and hydrophilized PLGA/PVA (5 wt%) scaffolds was compared by the culture of human chondrocytes in the scaffolds, it was observed that the PLGA/PVA scaffold has better cell adhesion and growth than the control PLGA scaffold. From *in vivo* evaluation of tissue compatibility by the implantation of the scaffolds into the skull defects of rabbit, it was also observed that the PLGA/PVA (5 wt%) scaffold shows better bone regeneration inside the scaffold than the control PLGA scaffold.

In this study, we evaluated *in vitro* and *in vivo* degradation behaviors of the hydrophobic PLGA and hydrophilized PLGA/PVA (5 wt%) scaffolds. For the study of their *in vitro* degradation behavior, the scaffolds were immersed in phosphate buffered saline solution (PBS). To see the effect of solution pH on the degradation behavior of the scaffolds, the scaffold-immersed buffer solution was changed everyday (to keep solution pH constant) or was not changed until the experiment was ended (pH is continuously lowered by the acid formation owing to the degradation of PLGA). The degradation behavior of the scaffolds in both solutions (constant pH and decreasing pH with time) was compared in terms of the changes in dimension, molecular weight, mechanical properties (maximum load and modulus), and morphology of the scaffolds. The degradation behavior of the scaffolds was further investigated *in vivo* using a rat model. Since PLGA is hydrophobic, the porous scaffold fabricated with this polymer is not wetting or absorbing with the cell culture medium or body fluid. Usually the scaffolds are pre-wetted with ethanol and then exchanged with culture medium or saline solution before cell culture or implantation into body [15]. The PLGA and PLGA/PVA scaffolds with and without pre-wetting treatment were subcutaneously implanted into rats and the changes in molecular weight of scaffolds were compared.

Experimental

Materials

PLGA (lactic to glycolic acid mol ratio, 50:50) was purchased from Boehringer Ingelheim (Germany). Weight average molecular weight of PLGA determined by gel permeation chromatography (GPC; Waters Model SP8810, USA) using polystyrene standards (Polysciences, USA) was about 110,000. PVA (Mw, 15,000; 86–89% hydrolyzed) as a hydrophilic additive was purchased from Fluka Chemical Co. (Germany) and used without further purification. All other chemicals were analytical grade and were used as received. Water was ultra-pure grade (<8 mΩ) supplied from a Milli-Q purification system (USA).

Scaffold fabrication

Porous PLGA/PVA scaffold with PVA composition, 5 wt% was fabricated by a melt-molding particulate-leaching method, as described elsewhere [14]. Briefly, the scaffold was fabricated using PLGA/PVA blend sheet prepared by the thermal compression (30 MPa at 80 °C) of homogeneous mixtures of PLGA and PVA (5 wt%) fine particles which were freezed and crushed in a liquid nitrogen-containing freezer mill (SPEX 6700, Metuchen, USA). The prepared sheet ($\sim$150 μm thickness) was cut into discs with 16 mm in diameter. The PLGA/PVA disc was placed into a brass mold (18 mm diameter and 2.5 mm thickness) covered with 0.9 g sodium chloride salt particles (sieved to sizes between 200 and 300 μm) at the bottom and was covered at the top again with 0.9 g salt particles. Then the mold was thermally compressed under 20–30 MPa at 180 °C for 1 min and then the following compression under 50–60 MPa at 180 °C for 30 s. After taking out from the mold, the salt-containing disc scaffold was immersed in water for 6 h to leach out salts from the scaffold. Then the scaffold was freeze-dried and was sterilized before use by exposure to ethylene oxide. The PLGA scaffold as a control was also fabricated by the same method above except the addition of PVA during the sheet preparation. Surface and cross-section morphologies (porous structures) of the prepared PLGA and PLGA/PVA scaffolds were observed by a scanning electron microscope (SEM; Model 2250 N, Hitachi, Japan) operated at an accelerating voltage of 15 kV. The cross-sectional samples were prepared by fracturing the scaffold after being frozen in liquid nitrogen. Before morphology observations, the scaffold samples were coated with platinum using a sputter coater (SC 500 K, Emscope, UK) under argon atmosphere.

In vitro degradation test

Before *in vitro* degradation experiments, the control PLGA scaffold was pre-wetted by immersion in ethanol for 1 h and then exchanged with PBS (pH, 7.4) for 1 h since the PLGA is hydrophobic and thus the scaffold can not be directly immersed in the buffer solution. The PLGA/PVA (5 wt%) scaffold was easily wetted and immersed in PBS without any pre-wetting treatment owing to its hydrophilic character. The PLGA and PLGA/PVA

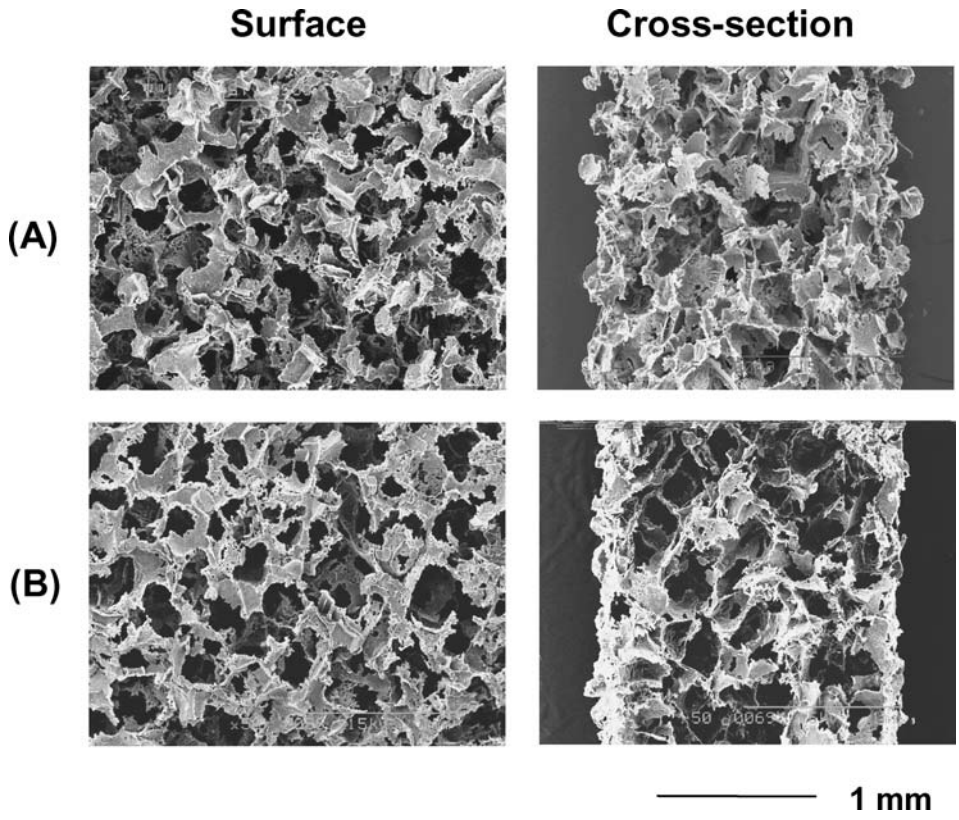

Figure 1 SEM photographs of (A) PLGA and (B) PLGA/PVA (5 wt%) scaffolds.

scaffolds were immersed in glass vials containing 15 ml of PBS solution and were placed in a 37 °C incubator for up to 70 days under static conditions. The samples were divided into two groups to compare their degradation behavior. In Group 1 the scaffold-immersed PBS solution was changed every day and in Group 2 the PBS solution was not changed until the experiment was ended.

The changes in pH (in PBS), dimension, molecular weight, mechanical properties (maximum load and modulus), and morphology of the scaffolds were examined with time. The pH changes of the PBS solution were measured using a pH meter (Model 350, Mettler-Toledo, UK). The dimensional changes of the scaffold in PBS were examined by the direct reading of a measuring scale attached on the outside bottom of the vial. The changes in molecular weight of the scaffolds were measured by GPC using chloroform as an eluent. The changes in mechanical properties of the scaffolds were measured by a biaxial tensile test equipment designed by our laboratory for small scaffold samples [14], which was attached in an Instron machine (AG-5000G, Shimadzu, Japan) with a 5 kg_f load cell. A rod with a ball-shape (diameter, 6 mm) tip was hammered vertically at a crosshead speed of 1 mm/min on the scaffold samples placed on a mold-type grip, and the load-displacement curves were obtained from the scaffold samples.

In vivo degradation test

The degradation behavior of the hydrophobic PLGA and hydrophilized PLGA/PVA scaffolds was further investigated *in vivo* using a rat model. The samples were divided into two groups to compare their degradation behavior. In one group, both PLGA and PLGA/PVA (5 wt%) scaffolds were directly implanted without pre-wetting treatment, even though the PLGA scaffold is not wetting or absorbing with body fluid. In the other group, both scaffolds were pre-wetted in ethanol for 1 h followed by immersion in normal saline for 1 h under sterile condition for the comparison, even though the PLGA/PVA scaffold is easily wetting with body fluid without pre-wetting treatment. The PLGA and PLGA/PVA scaffolds with and without pre-wetting treatment were subcutaneously implanted in the back of male Sparuge-Dawley rats (200–250 g) (two scaffolds in each group per rat). The scaffolds were harvested after 2 and 4 weeks of implantation and the molecular weights of the scaffolds were measured by GPC after dissolving the specimens in chloroform.

Results and discussion

Scaffold characterizations

PLGA and PLGA/PVA (5 wt%) scaffolds were fabricated by a melt-molding particulate-leaching method.

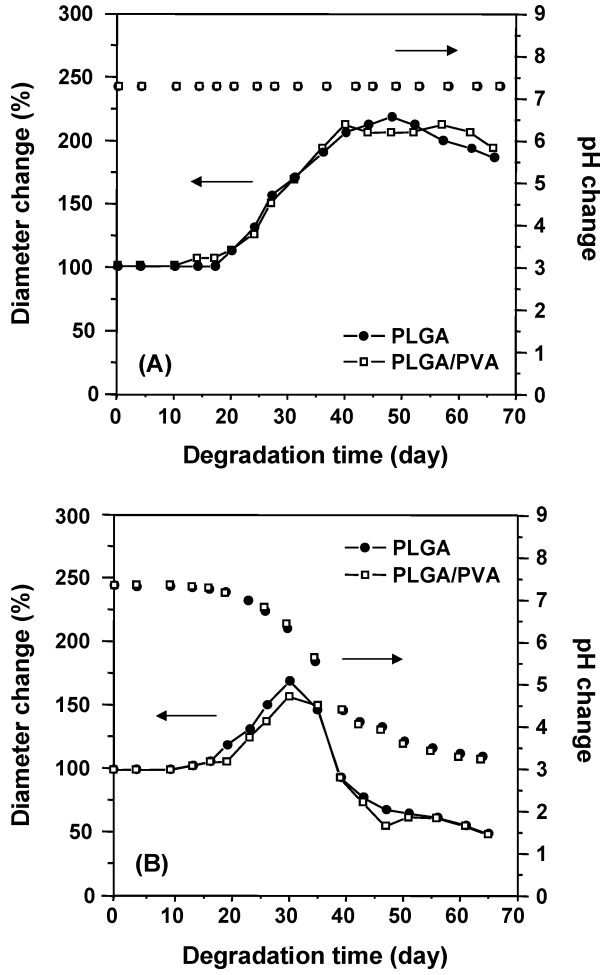

Figure 2 The changes in pH of PBS solution and diameter of PLGA and PLGA/PVA scaffolds with degradation time. (A) Group 1 (PBS change every day) and (B) Group 2 (PBS no change).

One benefit of our scaffold fabrication method is that it does not involve any organic solvents during the scaffold fabrication process. It is recognized that organic solvents are hard to completely remove from the scaffolds during the drying process and the organic solvent residue in the scaffolds may be harmful to cells or nearby tissues when implanted in the body [13]. PVA was used as a hydrophilic additive to prepare hydrophilized PLGA scaffold for easy wetting in cell culture medium or body fluid. The PVA in the scaffold was not easily leached out into cell culture medium or body fluid owing to its insolubility in water at room or body temperature (this polymer is soluble above about 70 °C [14]). The prepared PLGA and PLGA/PVA scaffolds exhibited highly porous and open-cellular pore structures with almost same surface and interior porosities (Fig. 1). This indicates that the salt particles were homogeneously distributed in the melted polymer matrix and the skin layer was not formed since the solvent was not used in this method. The pore sizes in the scaffolds were almost the same as those of added salt particles (sizes range from 200 to 300 μm). The porosity of the scaffolds determined by a mercury intrusion porosimetry was about 90%, which provides a surface area for cell-polymer interactions, sufficient space for extracellular matrix regeneration, and minimal diffusion constraints during *in vitro* cell culture [16, 17].

In vitro degradation behavior

When the scaffold-immersed buffer solution was changed every day (Group 1), the pH of the solution was kept to 7.4 with time (Fig. 2(A)), while the pH of the solution in Group 2 (no change of buffer solution) was remained constant for the first 14 days and then decreased significantly for both PLGA and PLGA/PVA scaffolds, owing to the acid formation during the degradation of PLGA (Fig. 2(B)). The pH dropped down to 3.2–3.3 after 65 days for both scaffolds. Once the scaffold placed in PBS solution, water penetrates into the scaffold pores, leading to hydrolytic cleavage of backbone ester bonds [18]. Each ester bond cleavage forms a new carboxylic acid end group, resulting in the solution pH drop and the enhanced intramolecular depolymerization by the increment of chain hydrophilicity [19].

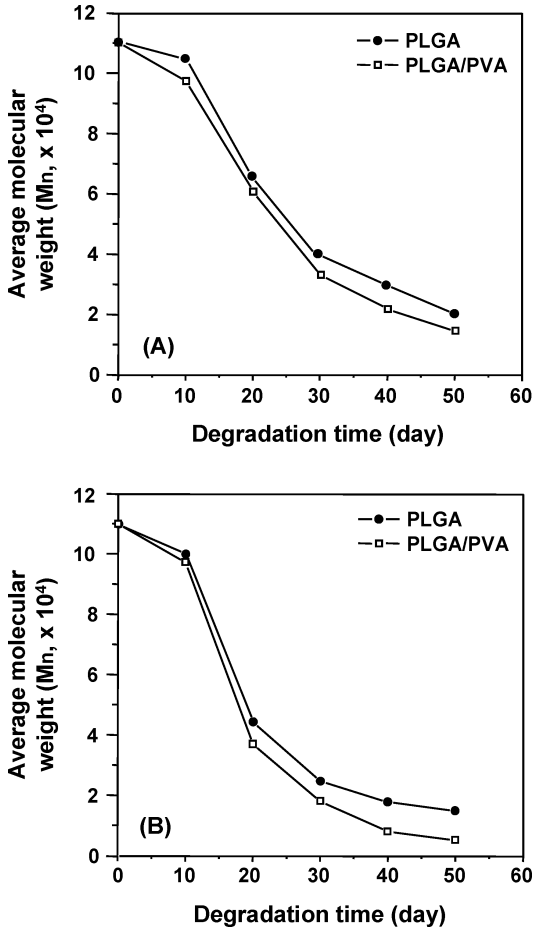

Figure 3 The changes in molecular weight of PLGA and PLGA/PVA scaffolds with degradation time. (A) Group 1 (PBS change every day) and (B) Group 2 (PBS no change).

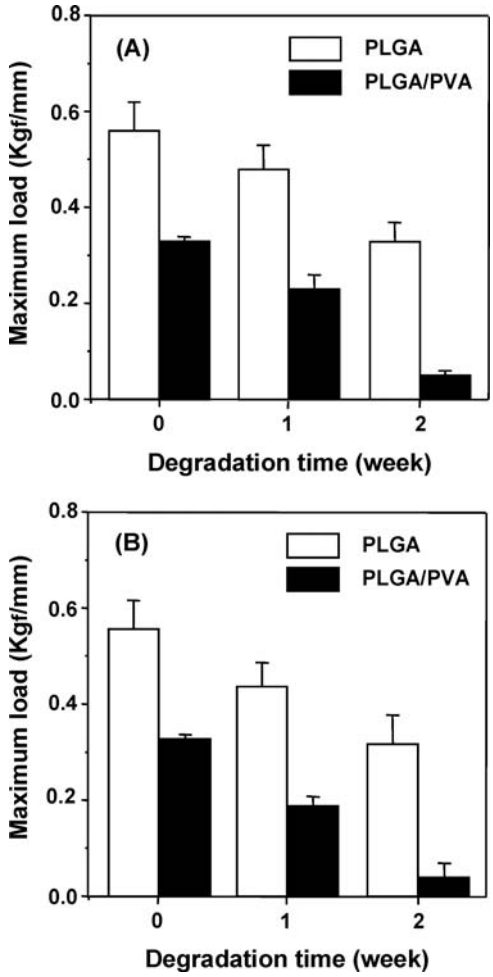

Figure 4 Comparison of maximum load of PLGA and PLGA/PVA scaffolds with degradation time. (A) Group 1 (PBS change every day) and (B) Group 2 (PBS no change) ($n = 5$).

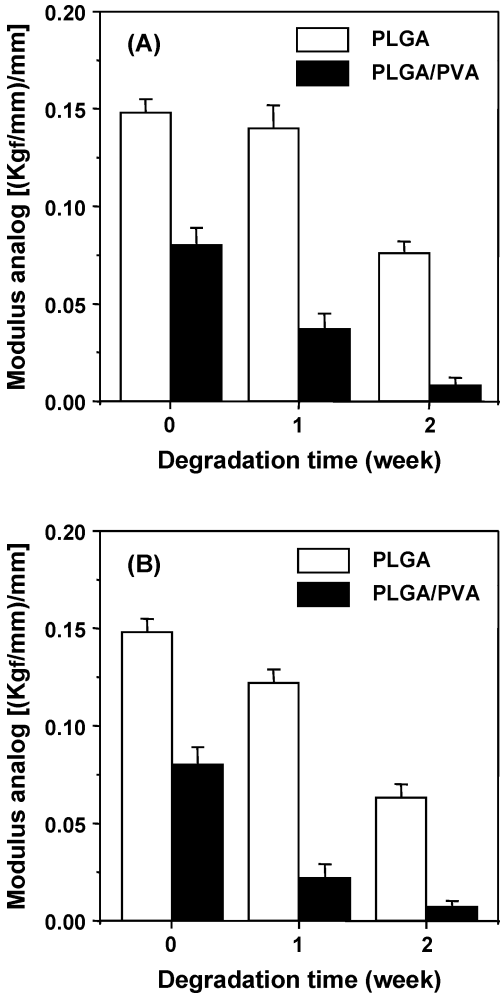

Figure 5 Comparison of modulus analog of PLGA and PLGA/PVA scaffolds with degradation time. (A) Group 1 (PBS change every day) and (B) Group 2 (PBS no change) ($n = 5$).

The scaffolds in two groups (Group 1, constant pH; Group 2, pH decrease) showed different dimensional changes in the solution with time. The scaffolds in both groups started to swell (by water uptake) in PBS solution after about 15 days (same period as the solution pH drop derived from the cleavage of ester bonds). The scaffolds in Group 1 showed more swelling than those in Group 2. The cleavage of PLGA backbone in the scaffolds gradually produces short-chain species that progressively can absorb more water molecules into the pores, resulting in the increased swelling of the scaffolds with time. The swelling of the scaffolds continued for 50 days in Group 1 (up to 200–230%) and for 30 days in Group 2 (up to 160–170%), and then the scaffolds started to de-swell in both groups. It was reported that de-swelling (or shrinkage) of PLGA scaffolds fabricated by organic solvent-containing conventional methods may be due to the microscopic motion of the polymer chains facilitated by the presence of residual solvent [20]. The PLGA and PLGA/PVA scaffolds fabricated by a melt-molding particulate-leaching method in this study do not involve any organic solvents during the scaffold fabrication process, and thus the effect of residual solvent on the de-swelling of PLGA scaffolds should be excluded. The de-swelling of the scaffolds shown in Fig. 2 may be due to physical disintegration and fragmentation of the scaffolds. They were collapsed after about 65 days. The existence of PVA in the PLGA scaffolds did not affect the dimensional change of the scaffolds in both groups, indicating little miscibility (phase separation) between PLGA and PVA. If PLGA and PVA blend is miscible (homogeneously blended), the resulting PLGA/PVA scaffold is expected to show higher swelling behavior owing to the hydration effect of PVA [21].

The changes in molecular weight of the scaffolds with degradation time were measured by GPC after dissolving the scaffold specimens in chloroform. The scaffolds in both group showed decreased molecular weights (sharply after about 10 days) owing to the cleavage of PLGA backbone ester bonds, however, the scaffolds in Group 2 (PBS no change and thus pH decrease) showed

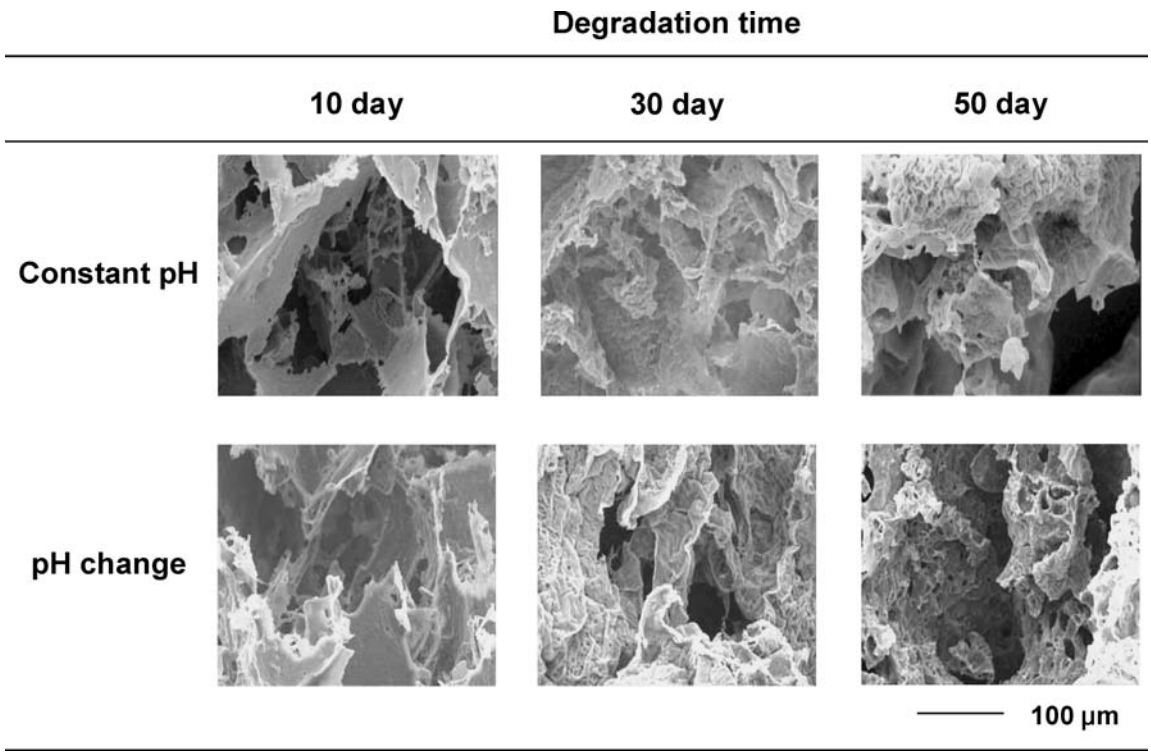

Figure 6 SEM photographs showing the cross-sectional morphologies of PLGA/PVA scaffolds with degradation time. (A) Group 1 (PBS change every day) and (B) Group 2 (PBS no change).

faster degradation than those in Group 1 (PBS change every day and thus no pH change) (Fig. 3). It seems that the carboxylic acids produced by the cleavage of backbone ester bonds of PLGA act as a hydrophilizer as well as a catalyst and thus accelerate the degradation of the scaffolds in Group 2 [18]. The hydrophilized PLGA/PVA scaffold showed slightly faster degradation behavior than the hydrophobic PLGA scaffold in both groups, probably owing to the easier water penetration into the scaffold matrix, even though PVA seemed to be heterogeneously blended with PLGA. The changes in mechanical properties of the scaffolds in both groups were also measured by a bi-axial tensile test equipment. From the load-displacement curves, we obtained the maximum load and modulus analog (from the initial slope of the load-displacement curve) values of the scaffolds (Figs. 4 and 5). The PLGA/PVA scaffold showed lower mechanical strength and modulus values than the PLGA scaffold. Both scaffolds showed the decreased mechanical strengths and became flexible with time owing to the decreased molecular weights, and the changes were more significant for the scaffolds in Group 2 (pH change) than in Group 1 (constant pH), as expected.

Fig. 6 shows the cross-sectional morphologies of the PLGA/PVA scaffolds during degradation in PBS solution (The PLGA scaffolds showed a similar trend; not shown). As the degradation was proceeded, the pore structures of the scaffolds in both groups became blunt and tiny holes in the scaffold matrix were produced, probably owing to the leaching or dissolution of low molecular weight PLGA fragments as well as PVA.

In vivo degradation behavior

Fig. 7 compares the changes in molecular weight of the PLGA and PLGA/PVA scaffolds subcutaneously implanted in rats with degradation time. After 2 weeks of implantation, both scaffolds (with and without pre-wetting treatment) showed the large decreases in molecular weight compared to the scaffolds before implantation (mol. wt, about 110,000). The decrease in molecular weight after 2 weeks of implantation was faster in the hydrophilized PLGA/PVA scaffold than the hydrophobic PLGA scaffold. In both scaffolds, the pre-wetted ones showed faster decrease in molecular weight than the non-wetted ones, probably due to the increased hydrolysis of the polymer contacting with water (body fluid). The non-wetted PLGA/PVA scaffold showed a similar molecular weight to the wetted PLGA scaffold (about 20,000). After 4 weeks of implantation, the scaffolds were highly degraded and the differences in molecular weight among the scaffolds (PLGA vs. PLGA/PVA, pre-wetted vs. non-wetted) were not significant. As the changes in molecular weight of the scaffolds were compared for *in vitro* and *in vivo* conditions (Figs. 3 and 7), the degradation of the scaffolds was faster in *in vivo* condition than *in vitro* condition for both PLGA and PLGA/PVA scaffolds. This phenomenon can be explained by the acceleration of

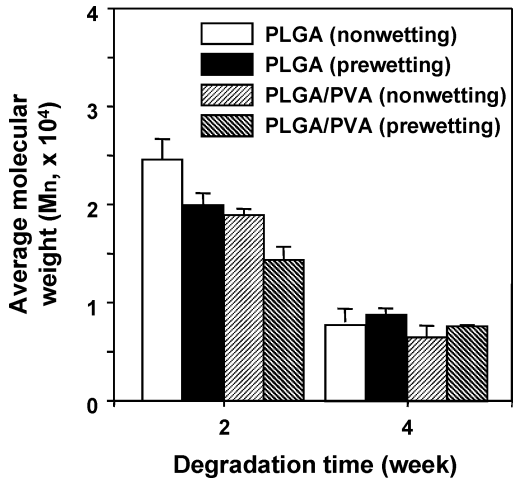

Figure 7 The changes in molecular weight of PLGA and PLGA/PVA scaffolds with implantation time (subcutaneous implant in rats) ($n = 3$).

PLGA degradation by foreign body giant cells or several enzymes in the body [19] as well as autocatalytic effect of the acidic degradation products accumulated locally in the medium surrounding the implant [22].

From this study, we can conclude that the surrounding conditions, i. e., with/without acid accumulation in the medium, scaffold pre-wetting/non-wetting, *in vitro* or *in vivo* condition, etc., affect sensitively the degradation behavior of the PLGA scaffolds. The hydrophilization of PLGA scaffold by the addition of PVA also affected *in vitro* and *in vivo* degradation behavior of the scaffold. A small amount of PVA addition (5 wt%) to fabricate PLGA/PVA scaffold was affected the hydrophilicity and *in vitro* and *in vivo* degradation behavior (faster degradation than hydrophobic PLGA scaffold).

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