A Study on the Effect of Degradation Media on the Physical and Mechanical Properties of Porous PLGA 85/15 Scaffolds

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Abstract: This study investigates the effect of PLGA 85/15 scaffold on the cell growth and viability of a cell line, and the degradation of the scaffold in different media. The cell line used was human promyelocytic leukemia cells (HL-60). Three different media were considered: distilled water, a phosphate buffered saline (PBS) solution, and HL-60 cell line. Porous PLGA 85/15 scaffolds were prepared with an optimized gas foaming/salt leaching technique using a NaCl/polymer mass ratio of five, a saturation pressure of 5.52 MPa and a saturation time of 12 h. The cell growth and viability were not impaired by the presence of the scaffold. The mass change of the scaffold due to degradation over the period was varied only by 4% across all three media. The average macropore size and molecular weight decreased as the degradation time increased in each medium. The scaffolds maintained mechanical and structural integrity throughout the study in all three media over the degradation period studied, and the change of Young's modulus of the scaffold under wet condition was not significant. Overall, PBS solution most strongly affected physical and mechanical properties, followed by dH_2O and HL-60 cells. The distinct variations of the scaffold's properties using different media, demonstrated the importance of carefully selecting the medium to perform *in vitro* studies. The medium must replicate the actual environment where the scaffold would be used, to represent accurately the changes in properties that the scaffold would be undergoing. © 2009 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 91B: 876–886, 2009

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INTRODUCTION

In situ biodegradable implants or scaffolds must maintain mechanical properties, morphology, and structure until the presence of the implant or the scaffold is no longer required. Adequate characterization of the biocompatibility, the degradation and the mechanical properties of biodegradable polymer is therefore essential in tissue engineering applications and implant design. Biodegradable polymers have the characteristic of degrading over time, requiring constant monitoring of the mechanical properties. For example, the variation in the strength of the implant or the scaffold must occur at the rate at which the tissue becomes increasingly capable of withstanding loads. The degradation of the polymer is influenced by the hydrophilic/hydrophobic characteristics of the polymer, the chemical composition, the configurational structure, the processing history, the molar mass, the polydispersity, the environmental conditions, the crystallinity, the morphology, the chain orientation, and the size of the matrix, to name a few.[1–3] Degradation studies are generally carried out using either *in vivo* or *in vitro* experiments. The *in vivo* experiments are usually performed in rabbits, sheep or rats at different body locations depending on the applications being studied.[4–9] The *in vitro* experiments are usually executed in media ranging from distilled water to more representative environments such as a saline solution or human cells lines.[4–6,8–17] The properties monitored as a function of time are (1) mass change,[5–13,15–17] (2) mass of water uptake,[5,6,12] (3)

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volume or dimension change,[5,6,9,11–15] (4) pore size change,[5,12] (5) molecular weight and polydispersity change,[4–6,8,10–13,15] (6) porosity change,[5,10,12,13] or (7) mechanical properties change.[4–6,9,10,12–15,17]

Despite the vast amount of research on porous material, monitoring and controlling the degradation rate of a scaffold or of an implant still requires significant research efforts. A thorough understanding of the physical features and the mechanical properties as a function of the degradation rate has also not been achieved. Consideration of mechanical testing in a wet environment, which replicates a more representative *in situ* environment of biodegradable polymers, has also been overlooked. Such an understanding of all these critical factors is essential to fabricate an implant, or to recreate a damaged tissue using the tissue engineering approach.

Studies have been conducted on biodegradable polymers scaffold made using the gas foaming/salt leaching technique.[18–20] However, there is limited information available regarding the effects of the porous scaffold on human cell lines and the effect of degradation media on the scaffold's physical and mechanical properties. In our past study[21] we evaluated the effect of the processing parameters on the scaffolds properties and concluded that the optimal properties were obtained when a NaCl/polymer mass ratio of five was used in combination with a saturation pressure of 5.52 MPa and a saturation time of 12 h. This study reports the effects of PLGA 85/15 scaffolds fabricated using an optimized gas foaming/salt leaching technique on HL-60. HL-60 cell lines were selected because of their ability to survive during long periods of time in culture. Another reason why they were selected is that as white blood cells they make up a component of the human immune system, which is responsible for detecting and attacking foreign material.

This study also presents the effects of three different media on the PLGA 85/15 scaffold's physical and mechanical properties. The media considered are distilled water (dH_2O), a phosphate buffered saline (PBS) solution and HL-60 grown in a complete medium. The physical properties monitored are the scaffold's mass loss, water uptake, molecular weight distribution change, volume loss, and porosity change. The mechanical property studied is the Young's modulus in compression under wet conditions.

EXPERIMENTAL

Materials

For the scaffold fabrication, 85/15 poly(DL-lactide-*co*-glycolide) acid (Lakeshore Biomaterials, AL), which is more commonly known as PLGA 85/15, was used. PLGA 85/15 is an amorphous polymer that has a glass transition temperature between 50 and 55°C and a specific gravity of 1.27 g/mL. The fabrication of the scaffold also required sodium chloride (NaCl) (Fisher Scientific, ON, Canada), dH_2O, and CO_2 gas cylinders (Praxair, ON, Canada).

Human cell lines HL-60 [American type culture collection (ATCC), VA] were used for the cell viability and the degradation studies. The HL-60 were grown in complete medium [RPMI-1640 media (Invitrogen Canada, ON, Canada)], supplemented with 10% fetal bovine serum (Sigma, St. Louis, MO), 2 m*M* L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin (Invitrogen Canada, ON, Canada). In addition, PBS solution (SIGMA, MO) and dH_2O were used in the degradation study. The PBS solution was obtained by dissolving tablet in deionized water. One tablet dissolve in 200 mL of deionized water yielded 0.01 *M* phosphate buffer, 0.0027 *M* potassium chloride and 0.137 *M* sodium chloride, and a pH of 7.4. Before immersion in human cell line HL-60, the scaffolds were sterilized with ethanol (70%). Fluorescence microscopy technique used in cell counting and viability investigations required ethidium bromide, fluorescein diacetate and Hank's balanced salt solution (HBSS). Lastly, the gel permeation chromatography (GPC) technique used to get the average molecular weight required the scaffolds to be dissolved using tetrahydrofuran (THF) as the solvent.

Experimental Procedures

Scaffold Fabrication. Scaffold made from PLGA 85/15 were fabricated by an optimized gas foaming/salt leaching technique as described previously. In brief, PLGA 85/15 pellets and NaCl particles were grounded and sieved separately, the former yielding particles between 106 and 500 μm, while the latter produced particles in the 106–250 μm range. Subsequently, NaCl/polymer disks using a NaCl/polymer mass ratio of five were prepared using a 12.8 mm diameter KBr die. The die was compressed for 60 s at 22,240 N (5000 lbs) in a carver press. The total mass of the NaCl/polymer disks was held constant at 250 mg. These solid disks were then saturated with CO_2, at a saturation pressure of 5.52 MPa and a saturation time of 12 h. A thermodynamic instability was then created by rapidly releasing the CO_2 pressure in the vessel to the atmospheric pressure. NaCl/polymer samples were then placed in dH_2O for 48 h to dissolve NaCl content; a porous matrix was subsequently formed. The dH_2O was replaced every 24 h. The amount of NaCl removed from the samples was also verified by comparing the mass of the samples before and after leaching.

Scaffold Sterilization. Before the immersion of the PLGA 85/15 scaffold in the HL-60 cells, the PLGA 85/15 scaffolds were sterilized in 70% ethanol for 1 h. The scaffolds were then rinsed in dH_2O and incubated in an open sterile petri-dish under a bio-hood for 24 h to insure the complete removal of the ethanol.

HL-60 Cell Viability Study. An investigation of the viability of HL-60 cells grown in complete medium with and without the scaffold was carried out. Five scaffolds were

placed in tissue culture flasks containing 15 mL of medium with an initial HL-60 cell concentration of 2×10^5 cells/mL. A control containing only 15 mL of medium with an initial HL-60 cell concentration of 2×10^5 cells/mL was used as a benchmark to determine the effect of the PLGA 85/15 scaffold on the cell growth and viability. During the entire study, the six flasks were kept in an incubator at 37°C (5% CO_2). A cell count was performed every day for the first 9 days and every 3 days for the remaining period, for an overall period of 35 days.

The cells were counted using the fluorescence microscopy technique similar to that of Strauss et al.[22,23] Briefly, a dye working solution was prepared by adding 7.5 μL of fluorescein diacetate (5 mg/mL in acetone) and 50 μL of ethidium bromide (200 μg/mL in HBSS) to 1.2 mL of HBSS. Equal volumes (10 μL) of the dye and cell suspension were mixed. The cell concentration and viability were assessed at low magnification by fluorescence microscopy (Olympus BH2, Olympus Optical, Tokyo, Japan; 10× magnification, narrow band "IB" excitation interference blue filter cube). Under the fluorometric assay conditions, viable, metabolizing cells appeared green due to fluorescein diacetate conversion to fluorogenic products by intracellular esterase activity, while non-viable cells, which could not exclude the DNA-dye ethidium bromide, were red.[23] Every 3 days, the cells were diluted with fresh medium to keep the cell concentration between 2×10^5 and 1×10^6 cells/mL over the entire study. When necessary, part of the culture medium was removed from the flask so that the total volume never exceeded 180 mL.

***In Vitro* Degradation Study.** *In vitro* degradation was carried out in three different media: dH_2O, a PBS solution, and a HL-60 cells culture. The study was done according to the American society for testing and materials (ASTM) F1635-95.[24] Before the degradation study, all the scaffolds were weighed and measured to record initial mass, volume and porosity. The scaffolds were placed in a petri-dish containing 55 mL of either dH_2O or PBS solution, and they were maintained at 37°C in an incubator. The pH was monitored every week and did not present any change. New medium was added every 2 weeks to compensate for the loss of medium due to evaporation.

Scaffolds were removed after week 1 and week 2 and then every 2 weeks subsequently over a total period of 3 months. The retrieved scaffolds were immediately weighted to monitor the water uptake. The scaffolds degrading in the PBS solution were then rinsed in dH_2O to remove any residual from the medium. Scaffolds from both medium were placed in their respective petri-dish under a bio-hood to dry for 48 h. The weight and the physical dimensions of scaffolds were then measured to determine the mass, volume and porosity loss. The same scaffolds were also tested in compression under wet condition, observed under SEM and tested using the GPC technique to obtain the molecular weight distribution change.

For the degradation study in HL-60 culture, the scaffolds were placed in tissue culture flasks in a total medium volume of 20 mL and were incubated at 37°C (5% CO_2). The initial HL-60 concentration was 2×10^5 cells/mL. The concentration was kept between 2×10^5 and 1×10^6 cells/mL at all times by removing part of the culture and adding novel medium every 3 days. The total volume was kept constant over the entire study. A control consisting of a set of six scaffolds placed in complete medium without HL-60 was used to benchmark the effect of the HL-60 on the scaffold. The control was only removed at the end of the degradation study. For the HL-60 culture study, scaffolds were removed every week over a period of 1 month. Retrieved scaffolds were immediately rinsed in dH_2O and then placed in their respective petri-dish under a bio-hood for 48 h to dry. The weight and the physical dimensions of the scaffolds were recorded to determine the mass, volume and porosity loss. The scaffolds were also tested in compression under wet condition, observed under SEM, and tested to obtain the molecular weight distribution change.

Sample Characterization

Water Uptake, Mass Remaining, Volume Loss, and Porosity. The percentage of water uptake was obtained from Eq. (1) where the wet mass of the scaffold (m_w) was measured immediately after the scaffold removal from the medium and where the dry mass of the scaffold (m_d) was measured after the 48 h drying period of the scaffold. The percentage of mass remaining was obtained from Eq. (2) where the initial mass of the scaffold (m_i) was measured before immersion in the medium. The volume loss was obtained from Eq. (3) where the initial volume (V_i) was obtained by measuring the scaffold dimensions before degradation and where the dry volume (V_d) was obtained by measuring the scaffold dimensions after the 48 h drying period following the degradation period. The dimensions measured were the diameter and the thickness of the disk. Three measurements of the diameter and the thickness were recorded and averaged.

$$\% \text{ water uptake} = \left(\frac{m_w - m_d}{m_d}\right) \times 100 \quad (1)$$

$$\% \text{ mass remaining} = 100 - \left[\left(\frac{m_i - m_d}{m_i}\right) \times 100\right] \quad (2)$$

$$\% \text{ volume loss} = \left(\frac{V_i - V_d}{V_i}\right) \times 100 \quad (3)$$

The density of the scaffold (ρ^*) was obtained from Eq. (4), where the mass (m) and volume (V) were measured before and after each degradation time. The relative density (ρ_r) and porosity (P) of the samples were determined from Eqs. (5) and (6), respectively, where ρ is the density of the unfoamed polymer, which was provided by the supplier.

$$\rho* = \frac{m}{V} \quad (4)$$

$$\rho_r = \frac{\rho*}{\rho} \quad (5)$$

$$P = (1 - \rho_r) \times 100 \quad (6)$$

Average Molecular Weight Determination. A GPC system (Waters Corp. MA) equipped with a refractive index detector and three Waters Styragel columns used in series (10^3, 10^4, and 10^6 Å) was utilized to obtain the molecular weight distribution (MWD) change from the GPC curves of the scaffolds after degradation. THF was used as the eluent at a rate of 0.3 mL/min under a temperature of 30°C. The samples were first dissolved in THF and filtered with a 0.45 μm PVDF filter before injection to avoid the introduction of high molecular weight gel. The injection loop volume available was 20 μL. Polystyrene standards were used to obtain a primary calibration curve. The values of the Mark-Houwink contants used to determine the average molecular weights of the scaffolds were $K = 1.07 \times 10^{-4}$ and $\alpha = 0.761$.[25] The analysis of the data was completed using the Empower 2 software (Waters Corp. MA)

Scanning Electron Microscopy (SEM). The pore morphology, pore size, pore density, and level of interconnectivity were evaluated using scanning electron microscopy. The samples were coated using a cold coating process by applying a thin layer of gold with the aid of a sputter coater (SEM Coating Unit PS3). The gas pressure was set at 2 KPa (20 mbar) and the current was applied at 9–10 mA; the entire coating time lasted 60 s. The edges of the coated samples and the SEM mounts were then painted with a conductive carbon paste. A JSM scanning electron microscope (Model 6060) was then operated at 20 kV, and images were acquired from several locations, at different magnification, on each sample. An SEM picture was taken for every degradation period and for every medium used.

Compressive Young's Modulus. After each degradation period, the compressive modulus of the scaffolds was acquired. The scaffolds were first pre-wetted in their respective degradation medium for 30 min in an incubator at 37°C before testing. The wet scaffolds were then tested at ambient temperature (25°C) using an Instron machine (Model 1122). The scaffolds were compressed between two plates with a constant deformation rate of 1 mm/min using a 500 N load cell. A small preload was applied to each sample before the compression test to ensure that the entire scaffold surface was in contact with the plates. The strain was calculated using the displacement of the crosshead and the compression modulus was determined from the slope in the elastic portion of the stress-strain curve. For each combination of parameters, five samples were tested and averaged.

RESULTS

Scaffold's Initial Physical and Mechanical Properties

The surface morphology of the scaffolds was observed with the aid of SEM pictures at magnifications of ×40, ×70, ×100, and ×300, as shown in Figure 1. The exterior surface of the scaffold presents an open and highly porous structure throughout the matrix. Two levels of pores are distinguishable: micropores, produced during the CO_2 pressure release, and macropores formed by NaCl leaching. The macropores are uniformly spread throughout the scaffold and the average macropore size is in the vicinity of 170 μm, which corresponds to the average size of the NaCl particles used in the fabrication of the scaffolds. The initial porosity, mass, and volume of the scaffold were 90%, 0.04 g, and 0.35 cm^3, respectively. The average diameter and thickness of scaffold were 14.5 mm and 2.1 mm, respectively. Finally, the initial Young's modulus in compression under wet conditions was 0.744 MPa.

Effects of PLGA 85/15 Scaffold on the Viability and the Growth of HL-60 Cells

Up to 5 days of incubation, there was no significant difference between the growth of HL-60 cells exposed and unexposed to the scaffolds. Only a slight increase in the viable cell number grown with the scaffold was detected after 5 days. Also, the effect of scaffold on the percentage of viable HL-60 cells was small over an incubation period of 30 days.

Effects of Degradation Media on the Physical Properties of PLGA 85/15 Scaffolds Change in the Scaffold's Mass, Water Uptake, and Average Molecular Weight

The degradation of PLGA 85/15 scaffolds in dH_2O and PBS solution for 84 days and in HL-60 cells for 28 days was investigated. For all degradation media, the mass loss of the scaffolds was within 4% throughout the full degradation period. Initially, the average molecular weight of the polymer matrix was uniform throughout the matrix.

Figure 2 shows the percentage of change in the water uptake of PLGA 85/15 scaffolds exposed to dH_2O and a PBS medium for a period of 84 days. The scaffolds water uptake in the PBS increased from 0 to 100% after 7 days and remained constant for the remaining of the degradation period. The water uptake of the scaffold exposed to dH_2O varied from 0 to 150% after 7 days of degradation and increased up to 200% towards the end of the degradation study. The water uptake profile was expected to increase with time, and then decrease as the scaffold lost mass.

Figure 3 shows the comparison in the MWD of the scaffolds degraded in the three different media after a degrada-

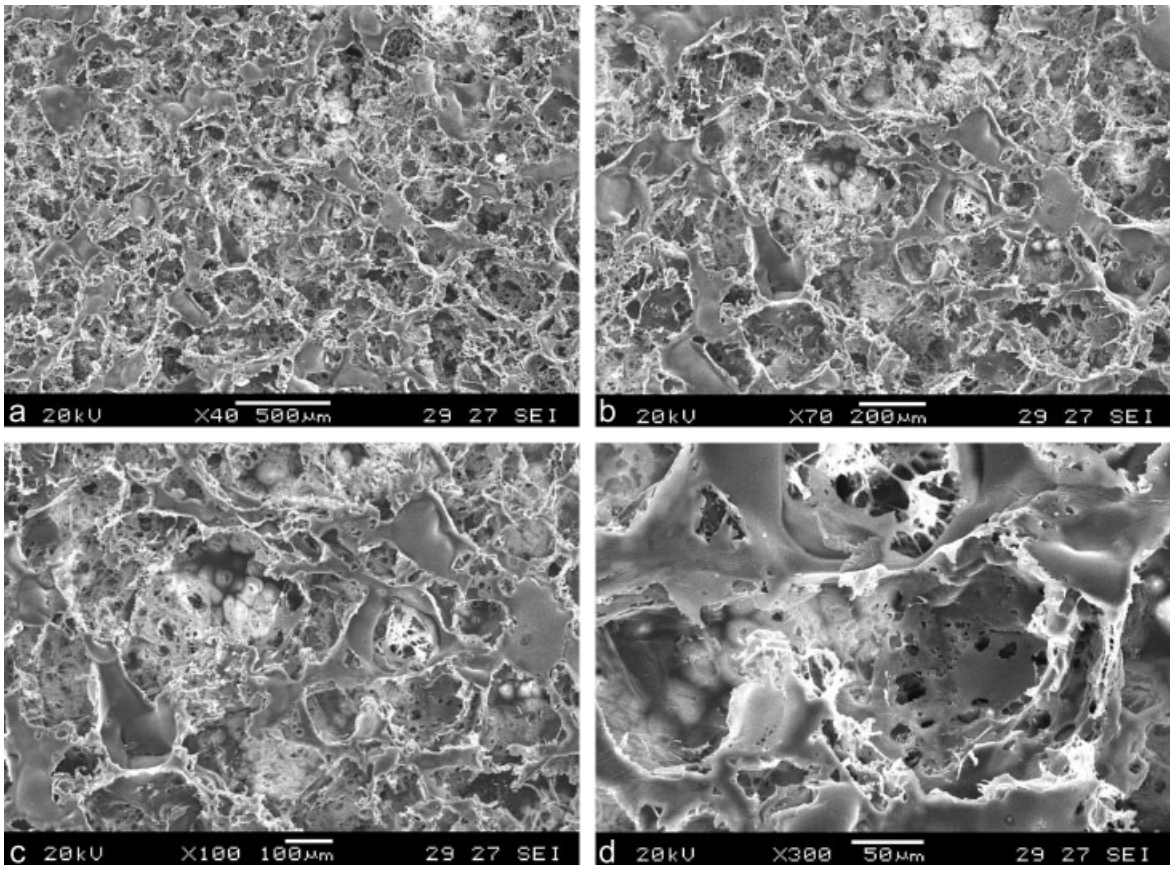

Figure 1. SEM images of the optimized PLGA 85/15 scaffold observed under magnification: (a) ×40, (b) ×70, (c) ×100, and (d) ×300 before being used in the degradation study.

tion period of 28 days. From Figure 3, it was observed that the peak from the PBS curves was shifted more to the left compared to the peak from dH_2O, which was shifted to the left more than the HL-60 peak. It can therefore be said that the PBS solution affected the average molecular weight most significantly, followed by the dH_2O and finally the HL-60 medium.

Figure 4 shows the MWD variation as the scaffolds degraded in different media. The MWD exhibits the expected single peak at low degradation time and the peak shifted to the left as the degradation increased. The peak shifting to the left indicates that the average molecular weight is decreasing as the degradation time increased in the three medium. It can also be observed

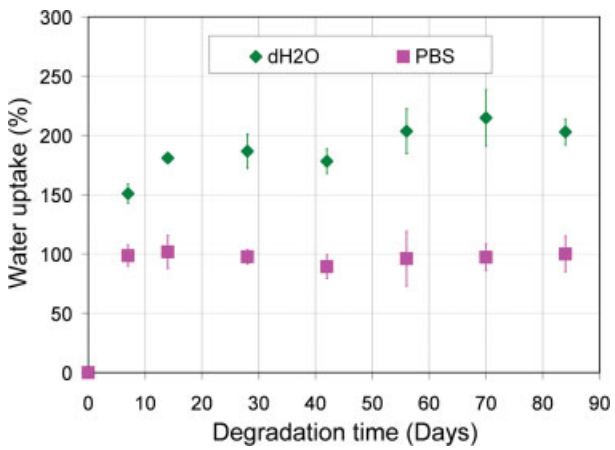

Figure 2. PLGA 85/15 scaffold's percentage of water uptake after degradation in different medium. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

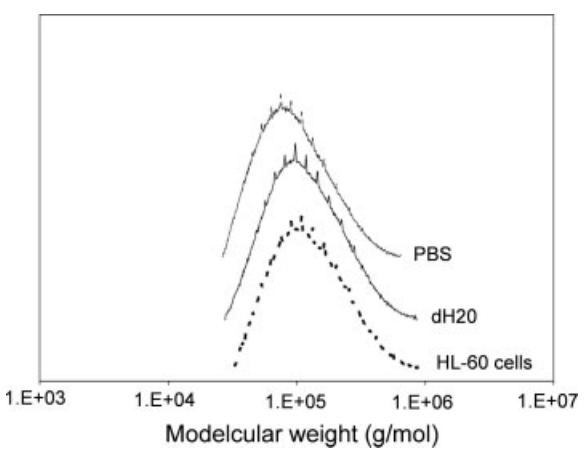

Figure 3. GPC curves of PLGA 85/15 scaffolds showing the effect of the different media on the MWD after a degradation time period of 28 days.

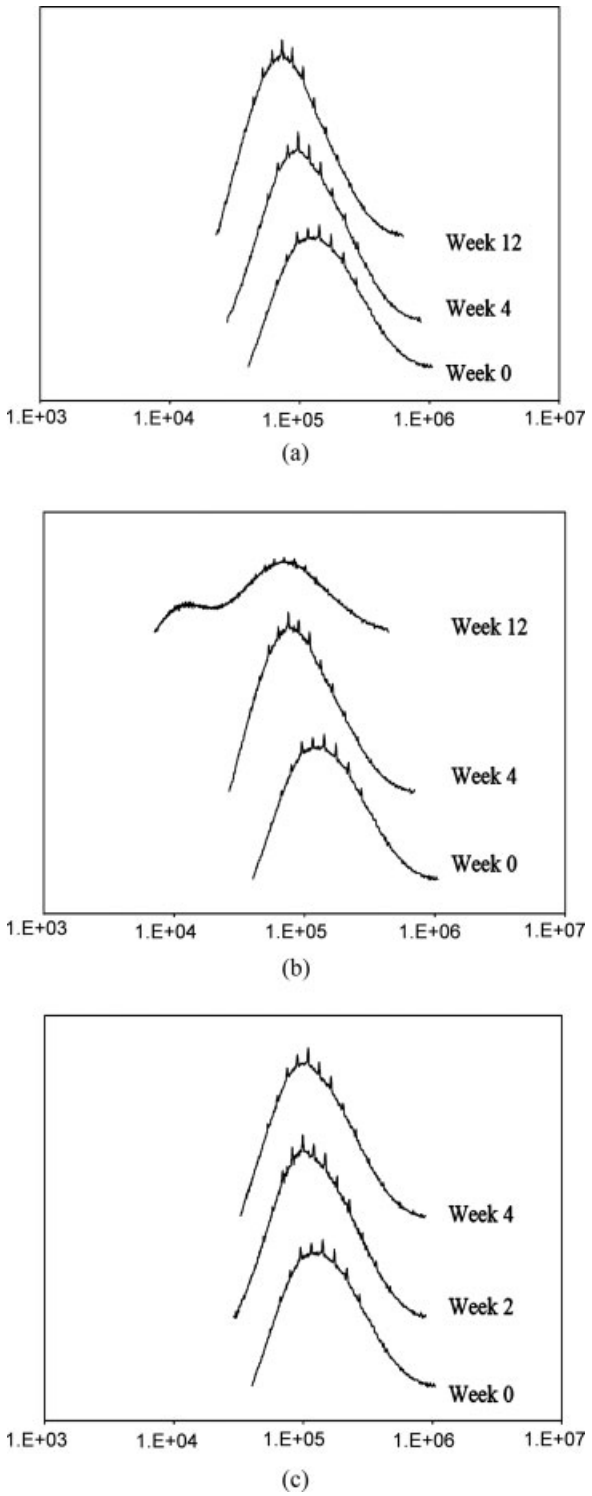

Figure 4. PLGA 85/15 scaffolds GPC curves showing the MWD after degradation period in: (a) dH_2O, (b) PBS solution, and (c) HL-60 cells.

from Figure 4 that as the degradation time increased, the MWD became very broad when the scaffolds were degraded in the PBS solution and manifested two peaks instead of a single one versus the MWD in the dH_2O and HL-60 cells.

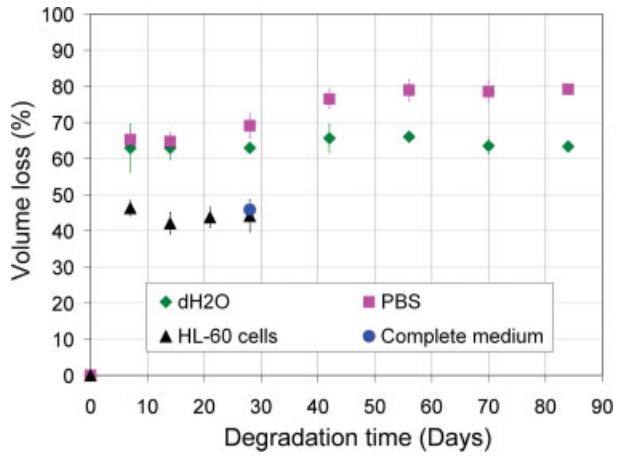

Figure 5. PLGA 85/15 scaffold's percentage of volume loss after degradation in different medium. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Change in the Scaffold's Dimensions and Morphology

Figure 5 shows the volume loss of PLGA 85/15 scaffolds degraded in dH_2O, a PBS solution and HL-60 cells. The volume loss of the scaffolds degraded in the PBS solution was 66% after 7 days of degradation, steadily increasing to 80% after 84 days. The volume loss of the scaffolds degraded in the dH_2O increased to 63% after 7 days of degradation and remained nearly constant for the remaining degradation period. The volume loss of the scaffold degraded in the HL-60 cells medium showed a loss of 46% and also stabilized for the remaining of the degradation period of 28 days.

Figure 6 presents a picture of the evolution of the degradation of PLGA85/15 scaffolds in the three different media. Visual observation of the samples indicates a degradation behavior similar to the volume loss characterization. The diameter of the scaffolds abruptly decreased within a degradation period of 7 days in all three different media. After 7 days, the diameter remained nearly constant for the remaining of the degradation period in dH_2O and in HL-60 cells. However, in the PBS medium, the diameter decreases slowly showing alternating increase or decrease due to the absorbing water. The largest diameter variation occurred for the PBS medium. The smallest variation of the diameter occurred in HL-60 cells.

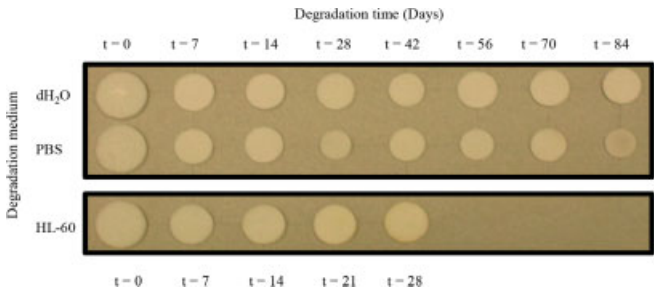

Figure 6. Photographs of PLGA 85/15 scaffolds degrade in different media. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

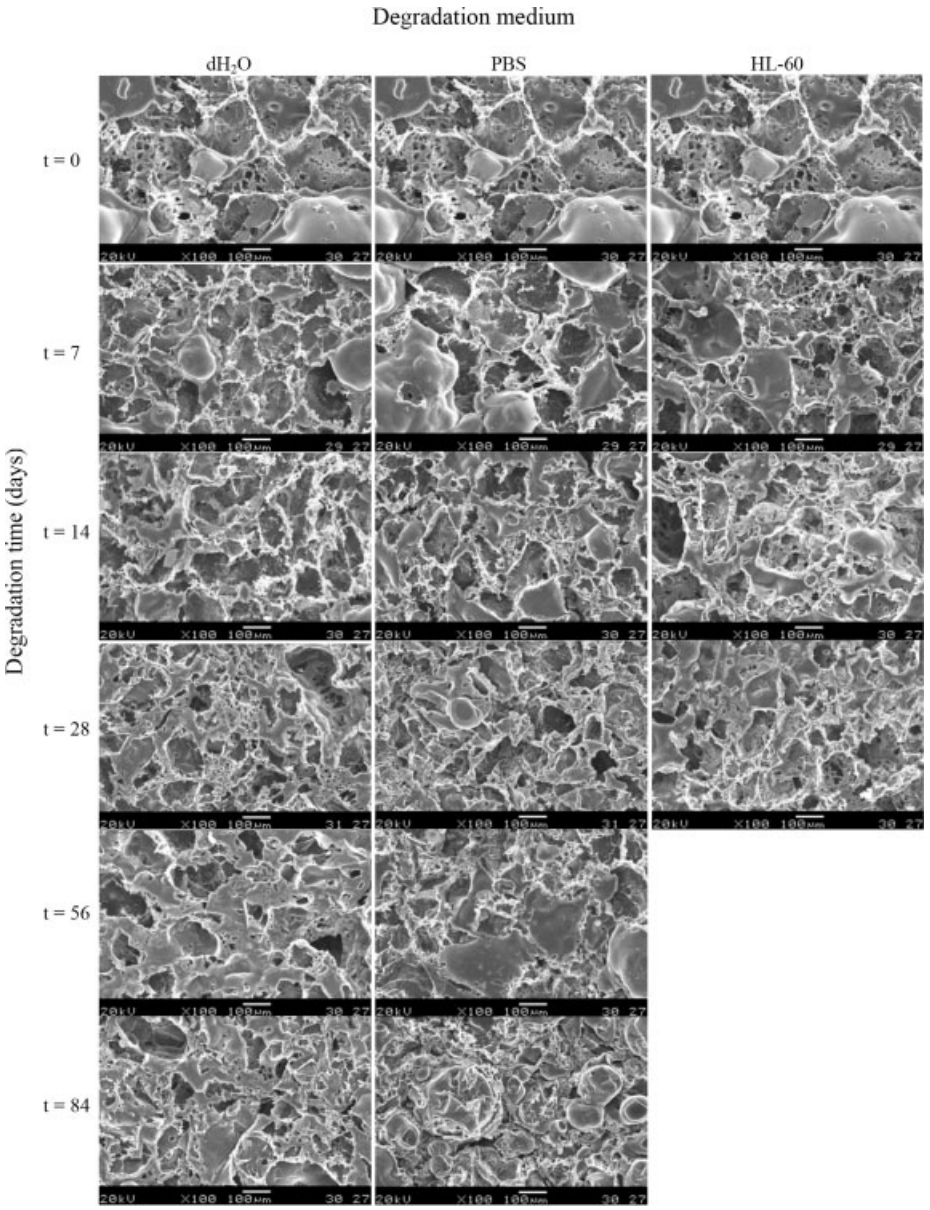

Figure 7. SEM images of PLGA 85/15 scaffolds observed under magnification ×100 after different degradation periods using three different media.

Figure 7 shows SEM pictures of the scaffolds degraded in different media at various degradation times period. Visual comparison indicated a larger original average macropore size than the average macropore size after a degradation period of 7 days, thus confirming that the sudden drop in volume was due to the shrinkage of the structure. Shrinkage of the macropore size was more significant in the PBS solution than other media. Furthermore, the shrinkage of the macropore size appeared to increase as the degradation time increased in all three media.

Figure 8 shows the change in porosity of scaffolds degraded in dH_2O and PBS solution for a degradation period of 84 days, and in HL-60 cells for an overall degradation period of 28 days. The largest decrease in porosity occurred in the PBS solution, varying from 90% to 72% after 7 days and thereafter decreasing to 58% after 84 days. The volume loss shown in Figure 5 and the evidence of shrinkage shown in Figures 6 and 7 while no change in mass is occurring, implicitly suggested a drop in porosity. The scaffolds degrading in dH_2O decreased in porosity, varying from 90% to 78% after 7 days of degradation and thereafter remaining relatively constant with a porosity of 74% after 84 days. The scaffolds degrading in HL-60 cells had the smallest change in porosity, achieving a porosity of 84% after the first 7 days of degradation. Similarly to dH_2O, the porosity of scaffolds degrading in HL-60 remained constant after the first week. Figure 8 also indicates no significant difference in porosity between the scaffolds immersed in HL-60 cells and in complete

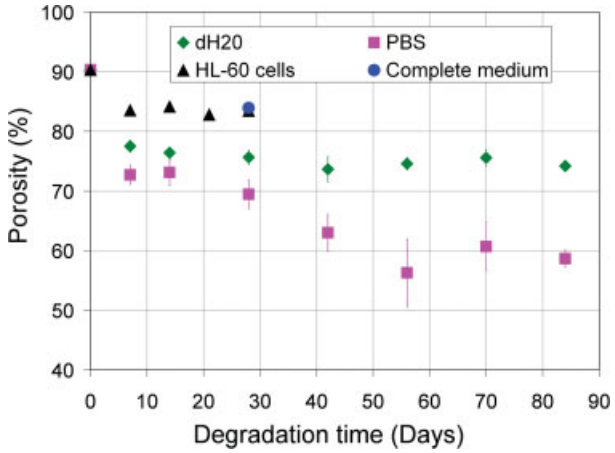

Figure 8. PLGA 85/15 scaffold's porosity change after degradation in different medium. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

medium only, suggesting that porosity was not affected by the presence of HL-60 cells.

Effects of Degradation Media on the Young's Modulus in Compression of PLGA 85/15 Scaffolds Tested in Wet Condition

Compressive tests under a wet condition were performed to evaluate the Young's modulus of the scaffold. A wet condition was chosen to more accurately represent the environment of utilization of the scaffolds. Compressive Young's modulus of PLGA 85/15 scaffolds degraded in dH_2O, a PBS medium, and HL-60 cells as a function of degradation time are illustrated in Figure 9. Figure 9 indicates that Young's modulus in compression decreased during the first 14 days from 0.744 to 0.13 MPa for the PBS solution and from 0.744 to 0.64 MPa for the dH_2O. For degradation period larger than 14 days, Young's modulus increased at a higher value in the PBS solution than in dH_2O. The scaffolds degraded in the HL-60 cells increased continuously from 0.744 to 0.96 MPa after 28 days.

DISCUSSION

Effects of PLGA 85/15 Scaffold on the Viability and the Growth of HL-60 Cells

The PLGA 85/15 scaffolds produced in the present experiment exhibited a highly interconnected pore network. The difference in the number of viable HL-60 cells between exposed and unexposed to the scaffolds after 5 days of incubation might be due to cells reacting to the presence of the synthetic material, but further investigations are required to understand the full nature of the response. Overall, the growth of HL-60 cells cultured with and without the scaffolds behaved similarly.

The percentages of viable cells decreased by 5–8% after an incubation time of 7 days, and after 2 weeks, the viability of cells further dropped to about 72%. The decreased viability might indicate that the environment became inadequate for cell growth as the culture period increased. Additionally, the number of passages performed by the cells is limited, after which the cells disintegrate. Higher viable cell content for the first 9 days of incubation versus the remaining incubation period might also be due to the mixing of the cells. During the initial 9 days, the cells were mixed daily during the cell count. Mixing intervals increased to 3 days for the remaining incubation time period. Mixing might have improved the transfer of nutrients to the cells as the cells tend to settle to the bottom of the flask when grown under static conditions. Thus, an increase in mixing interval period from 1 to 3 days might explain the drop in the percentage of viable cells after 9 days.

Effects of Degradation Media on the Physical Properties of PLGA 85/15 Scaffolds Change in the Scaffold's Mass, Water Uptake, and Average Molecular Weight

As the scaffold is introduced in the medium, water penetrates into the polymer, leading to hydrolytic cleavage of ester bonds without any change in the mass of the polymer. Each ester bond cleavage forms a new carboxyl end group that accelerates the hydrolytic reaction of the remaining ester bonds.[26] During this reaction, the polymer chains are retained within the matrix until the chains reach a critical molecular weight and become water-soluble. The chains exit the matrix once dissolved, and the mass begins to decrease. The degradation period studied might not have been long enough for the polymer chains to become water-soluble. Therefore no significant mass loss was observed after 28 days in HL-60 and 84 days in the dH_2O and the PBS medium.

As shown in Figure 2, water uptake would initially increase because the first mechanism occurring in degradation is the absorption of water. Following the absorption,

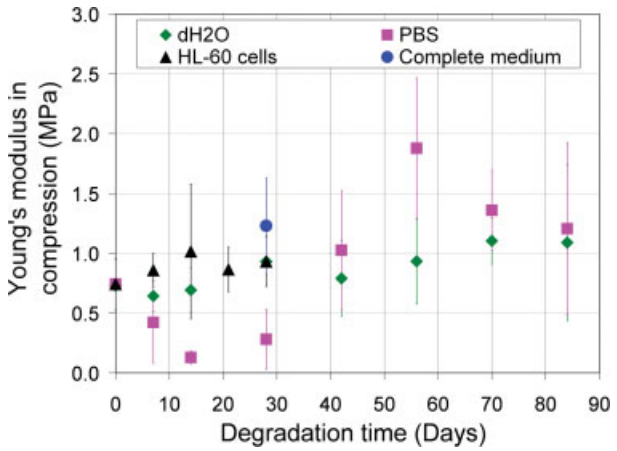

Figure 9. PLGA 85/15 scaffold's Young's modulus in compression tested under wet condition after degradation in different medium. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the chains of the polymer would start to shorten, due to cleavage of the polymer backbone. Once the chains become small enough and soluble in water, the scaffold would start disintegrating, which would cause a drop in the water uptake. Also observed is that the water uptake was lower than expected due to the ratio of PLA to PGA used, that is 85/15, corresponding to low PGA content.[6] The rate and the amount of water uptake increase as the content of glycolic acid increases in the copolymer, and conversely, lower water uptake and slower rate occur as the content of glycolic acid decreases. This behavior is due to the difference in the polymer backbone toward hydrolytic cleavage within the polymer structure. Therefore, a higher PGA content has greater hydrolytic susceptibility, which causes higher water uptake at a faster rate. The difference in water uptake between dH_2O and the PBS medium might be due to the chemical difference between the two media, resulting in the polymer backbone reacting differently to the two media. No subsequent drop in the water uptake was observed, since as shown above, no mass loss was observed for the period of degradation under consideration.

The difference of GPC curves in Figure 3 is due to the difference in chemical reactions of the scaffold with the medium, which in return affect the polymer backbone differently. From Figure 2, it can be stated that the polymer chains did not reach the critical length at the end of the degradation periods, since no significant drop in the mass and the water uptake were observed.

The drop in average molecular weight was expected as mentioned above due to the degradation mechanism occurring with PLGA. The MWD increases due to the amount of chains that are broken. As the average molecular weight of the chain decreases, it creates a higher number of chains, which also creates a larger range of chains lengths, therefore increasing the MWD.

Change in the Scaffold's Dimensions and Morphology

The volume loss of the control—that is the scaffold immersed in complete medium in the absence of HL-60 cells—was similar to the scaffold in the presence of HL-60 cells after 28 days (Figure 5), which implies that the presence of cells did not affect the volume loss of the scaffold. As shown in the figure, the volume loss increases significantly up to 7 days and changes slightly with time after that. The initial scaffold shrinkage is believed to be due to an increase in polymer chain mobility, and as a result, a decrease in glass transition temperature. The increase in chain mobility allows the oriented polymer chains in the expanded, gas foamed samples to relax at an elevated temperature of 37°C. As degradation progressed (between 7 and 14 days), the scaffolds absorbed more water, which causes the samples to swell and the volume loss in the figure looks decreased slightly.

The sudden decrease in volume from day 0 to day 7 as shown in Figure 6 might be due to the shrinkage of the structure inside the scaffold. Shrinkage might be due to the shorter polymer chains as the water uptake induces cleavage of the bonds. Shorter chains might change positions due to their smaller sizes and might induce an overall macroscopic shrinkage at first. The larger change in volume when the scaffold was degraded in PBS medium was due to the higher change in average molecular weight induced by the medium. Figure 6 also indicates that there was no physical disintegration, that is detaching sections of the scaffold, occurring during degradation for the period under investigation.

The various degradation responses of the scaffold in the three media might be attributed to the different chemical composition of each medium. For example, HL-60 cells medium induced the smallest change in volume loss. The reaction with the polymer backbone and HL-60 cells was slower than with the PBS solution. Figure 3, shows a smaller decrease in average molecular weight in the case of HL-60 compared to the PBS solution. The presence of different chemicals in the HL-60 cells medium, (e.g., proteins) might have limited the amount of water contacting the polymer link, and therefore the breakage of the backbone would occur at a slower rate.

Effects of Degradation Media on the Young's Modulus in Compression of PLGA 85/15 Scaffolds Tested in Wet Condition

The morphology of the matrix—that is the porosity, the pore size, the interconnectivity between the pores, and the orientation of the pores are all factors that significantly influenced the mechanical properties. Additionally, the density of the material and the degradation also affected the mechanical properties. In addition, macroscopic factors were strongly influenced by the microstructure, that is the length of the polymer chains. After 7 days of degradation, the porosity and the pore size decreased. Based on the latter two macroscopic parameters, Young's modulus would have been expected to increase. However, Young's modulus only increased when the degradation medium used was the HL-60 cells. For dH_2O and the PBS solution the increase in the Young's modulus was preceded by a decrease. Cleavage of the backbone linkages between the polymer repeating units may have caused the initial decrease in Young's modulus, overriding the effects of lower porosity and pore size on Young's modulus. This was not observed in the case of the HL-60 since the average molecular loss was smaller. A longer degradation time study would have been required to identify the time at which the scaffolds begin weakening due to the critical point, where the polymer chains start becoming soluble.

CONCLUSIONS

PLGA 85/15 scaffolds were successfully prepared by a gas foaming salt leaching technique. HL-60 cell growth and

viability exposed to PLGA 85/15, and the degradation behavior of PLGA 85/15 scaffolds have been investigated *in vitro*.

The HL-60 cell growth and viability in the presence of the scaffold were similar to HL-60 behaviors in the absence of the scaffold. Foreign material did not affect the cell growth for an incubation time of up to 7 days. It was also shown that the percentage of cell viability was not affected by the scaffold for a period of 35 days. A longer study period would have been required to observe the effects of material release from the scaffold (i.e. lactic and glycolic oligomers) in the HL-60 cells medium.

The degradation of the scaffold was performed in dH_2O, a PBS solution and HL-60 cells. The degradation process initially occurred when water absorption by the scaffold caused the breakage of the polymer chains. As a result, the molecular weight, the volume and the porosity of the polymer decreased. Smaller chains allowed the microstructure to be rearranged, leading to macroscopic shrinkage of the scaffolds. Throughout degradation, the mass of the matrix remained unaffected. However, extended degradation periods would have produced mass loss. A longer degradation period would have permitted larger amounts of water uptake, causing increased polymer chain cleavage. The molecular weight of the chains would have decreased down to the critical level. Once the average molecular weight of the polymer would have reached criticality, chains would have started to escape the matrix. The evidence of mass loss would then have been more explicit.

Overall, the PBS medium appeared to have the most significant impact on the physical properties of the scaffolds during the initial phase of degradation, and continuously affected the scaffolds along the full degradation period. The degradation in dH_2O and the HL-60 cells initially produced large variations in the scaffold's properties, but subsequent temporal changes were minor. The average macropore size decreased significantly in all three media. Research is thus required to study the effect of the decrease in the average pore size on tissue growth. Overall, the scaffold's properties were affected differently depending on the degradation medium. Thus, the choice of the medium employed for *in vitro* studies is critical to replicate the actual conditions experienced by the scaffold. For the periods under consideration, the scaffold kept its integrity and the Young's modulus in compression varied slightly. The scaffolds are thus potential candidate for tissue growth.

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