In vitro and in vivo degradability and cytocompatibility of poly(l-lactic acid) scaffold fabricated by a gelatin particle leaching method

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Abstract

Porous poly(l-lactic acid) (PLLA) scaffolds fabricated by a gelatin particle-leaching technique have good mechanical property and cytocompatibility, as demonstrated by a previous in vitro study. Here we investigate further the in vitro degradation of the scaffolds in terms of weight loss, water uptake, weight-average molecular weight, thermal behavior and morphology during a 39 week period in phosphate-buffered saline. The water uptake decreased dramatically during the initial stage due to release of the remaining gelatin, and then increased slightly with degradation time. The weight-average molecular weight decreased linearly as a function of time, while the crystallinity steadily increased with slightly decreased melting temperature. After degradation, many defects and big holes were seen in the scaffolds by scanning electron microscopy. Cartilage regeneration and scaffold disappearance in vivo were compared by implanting the construct into nude mice for 30–120 days. While the scaffolds maintained their intact pore structure after 23 weeks of degradation in vitro, they almost disappeared in vivo at the same time, implying a faster degradation rate in vivo. By 120 days after implantation, the scaffolds were hardly seen in the newly formed cartilage-like tissue. The regenerated cartilages could not maintain their predesigned shape after a long period of in vivo culture due to the weakening of the mechanical strength of the constructs as a result of PLLA degradation. The regions occupied initially by PLLA scaffold were filled later by collagen type II secreted by the chondrocytes, but with no evident basophilic proteoglycan.

Keywords: Poly(l-lactic acid) (PLLA); Porous scaffolds; Degradation; Tissue engineering

1. Introduction

In many tissue engineering applications, scaffolds with a porous structure are often desirable for the purpose of cell infiltration, extracellular matrix (ECM) production, vascularization and tissue ingrowth [1–4]. Porous poly(l-lactic acid) (PLLA) scaffolds have been widely used to guide the regeneration of cartilage [5–7], bone [8], skin [9], ligament [10], bladder [11] and liver [12] in a tissue engineering manner. It has been processed into porous scaffolds by a variety of methods, such as porogen leaching [13–17], phase separation [18–20] and electrospinning [21,22].

The success of aliphatic polyesters in tissue engineering relies largely on their degradability and biocompatibility, as well as their good processibility and mechanical properties. This is very promising since the foreign materials will eventually be removed from the body as the new tissues are formed [1–3]. In principle, the degradation rate of the scaffold should match the rate of tissue formation. Therefore, the degradation behavior of a scaffold has crucial impact on the long-term performance of a tissue-engineered cell/scaffold construct [23]. PLLA has been known to degrade by simple hydrolysis of the ester bonds into lactic acid, which is eventually removed from the body by the normal
2. Materials and methods

2.1. Materials

The PLLA (\(M_n = 99,000, M_w = 212,000\)) was synthesized using the method described previously [30]. Gelatin and 1,4-dioxane were obtained from Shanghai Chemical Industries Co. Ltd. A 10% (w/v) PLLA/1,4-dioxane solution was prepared for further use.

2.2. PLLA scaffold fabrication

PLLA was fabricated into porous scaffolds by a porogen-leaching technique with gelatin particles as the porogen [16]. Briefly, the gelatin particles (280-450 \(\mu\)m) sieved from raw gelatin were added into a glass mold, a cylindrical vial with a diameter of 22 mm. The vial was tapped gently to make a flat surface of the gelatin particles. The mixture was frozen at \(-25^\circ\)C for 3 h, then freeze-dried to remove the 1,4-dioxane. The PLLA porous scaffolds were obtained by leaching the gelatin assembly in 100 ml of deionized water at 70 \(^\circ\)C for 10 h.

2.3. In vitro degradation of the PLLA scaffolds

The scaffolds were cut into a 2×4×(8–10) mm shape with a razor blade. Each sample, of weight (\(W_0\)) ~ 15 mg, was immersed into 5 ml of phosphate-buffered saline (PBS) (pH 7.4; 0.8 g NaCl; 0.2 g KCl; 2.9 g Na2HPO4·12H2O and 0.2 g KH2PO4 were dissolved in 11 of distilled water). The samples in glass tubes were placed in a water bath at 37 \(^\circ\)C for up to 39 weeks. Every 2 days the buffer solution was replaced with 5 ml of fresh PBS. At a given time point, three samples were taken out and rinsed with distilled water three times. The surface water was adsorbed by a filter paper and the wet weight \((W_t)\) was measured immediately. These samples were subsequently air-dried at 30 \(^\circ\)C to a constant weight \((W_f)\) before being subjected to characterizations of molecular weight, thermal properties and morphology.

The weight loss and water uptake were defined as \((W_0 - W_t)/W_0 \times 100\%\) and \((W_t - W_f)/W_f \times 100\%\), respectively, according to Refs. [46,47]. Each result was the average of three parallel measurements, expressed as mean ± standard deviation.

2.4. Instrumental characterizations

The PLLA molecular weight was determined by gel permeation chromatography (GPC) (Waters 515). The samples were dissolved in tetrahydrofuran (THF) at a concentration of ~0.5%. THF was used as a flow phase at a flow rate of 1 ml min\(^{-1}\). The temperature was set at 40 \(^\circ\)C. The molecular weight and polydispersity index were obtained by referring to a calibration curve recorded from polystyrene standards (polysciences).

The thermal properties of the PLLA scaffolds, including melting temperatures and corresponding enthalpy changes, were measured by a Perkin–Elmer DSC 7 calorimeter. The samples (between 2 and 4 mg) were heated at a rate of 10 \(^\circ\)C min\(^{-1}\). The peak temperatures of melting endotherm were recorded as \(T_m\). The intrinsic degree of crystallinity \((X_c)\) was calculated by \(X_c = \Delta H_m/\Delta H_m^0\), where \(\Delta H_m^0\) is the melting enthalpy of the measured PLLA and \(\Delta H_m^0\) is the melting enthalpy of 100% crystalline polymer (203.4 J g\(^{-1}\)) [31].

To observe the morphology change under a scanning electron microscope (SEM), samples with different degradation time were coated with a gold layer under a pressure of 50 mtorr for 180 s. The microstructure of the scaffolds was then observed by SEM (JSM-5510LV, Japan).

2.5. In vitro chondrocyte seeding and culture

Chondrocytes were isolated from New Zealand rabbit ears (the rabbits were sacrificed under the institutional ethical guidelines) by digesting the cartilage chips with 0.2% collagenase II (Sigma) and cultured in Ham’s F-12 medium supplemented with 20% fetal calf serum (FBS), 300 mg l\(^{-1}\) glutamine, 50 mg l\(^{-1}\) vitamin C, 100 U ml\(^{-1}\) penicillin and 100 U ml\(^{-1}\) streptomycin. The cell suspension was then
seeded in 9 cm tissue culture dishes (Falcon, seeding density 2 × 10^6 cells cm^-2) and incubated in a humidified atmosphere of 95% air/5% CO_2 at 37 °C. After a confluent cell layer was formed (about 3–4 days), the cells were detached using 0.25% trypsin in PBS and resuspended in the supplemented culture medium as described above before being used for the experiments.

Before cell seeding, the PLLA scaffolds were sterilized using 75 vol.% ethanol for 1 h and then incubated in PBS for 1 day to exchange ethanol. Chondrocyte suspensions (2 × 10^6 cells ml^-1) were injected into the scaffolds using a syringe. The cell-containing scaffolds were then incubated in the culture medium under the conditions as described above. The cells seeded in all the specimens were obtained from the same donor.

2.6. In vivo histological investigation

The scaffolds seeded with chondrocytes in vitro for 7 days were implanted subcutaneously in the dorsum of athymic nude mice. To observe the cell distribution under confocal laser scanning microscopy (CLSM) before implantation, the cell-containing scaffold was taken out from the culture plate and rinsed gently with PBS. Then 5 μg ml^-1 fluorescein diacetate (FDA)/PBS solution was slowly injected into the scaffold. After incubated for 10 min the scaffold was gently cut into several parts. By this fluorescent staining, only the viable cells in the scaffold can be visualized under CLSM [48]. The cell content in the samples was assessed by quantifying DNA (7.7 pg DNA cell^-1) in the constructs using the Hoechst 33258 dye (Sigma) assay [49,50]. Hoechst 33258 is a bis-benzimide derivative that binds to the AT-rich regions of double-stranded DNA. It is excited in the near-UV (350 nm) spectrum and emits in the blue region (450 nm). Samples were frozen at −20 °C for 2 h, lyophilized for 24 h and digested with 1 ml of papain (1% w/v, with 0.09% EDTA (disodium ethylenediaminetetraacetic acid) as solvent) per sample. Shortly after 100 μl papain digest solution was added into 2 ml Hoechst 33258 solution (1 μg ml^-1), the fluorescence intensity at 450 nm was measured by a fluorescent spectrophotometer (RF-5301PC, Shimadzu). The DNA content of the samples was determined by referring to a calibration curve of standard DNA.

The athymic nude mice were purchased from Shanghai China for animal experiments. Athymic male mice were obtained at 5 weeks and acclimated for 1 week before use. Under anesthesia with ether vapor, two transverse incisions were made on the dorsum of each mouse to create a subcutaneous pocket using a sterile surgical technique. Each animal received low cell/scaffold constructs on the back (Fig. 5b). The cell–material constructs were harvested after in vivo cultivation for 30–120 days. Samples for histological evaluation were fixed in neutral buffered formalin, embedded in paraffin and sectioned in cross-section through the center of the implant (5 μm thick sections). The cross-sections were routinely stained with hematoxylin and eosin (HE) and Alcian blue (blue stain for glycosaminoglycan (GAG)) [45]. The sections were also used for the immunostaining of collagen type II by collagen type II antibody (PV-9000, Poly-HP anti-Mouse/Rabbit IgG Detection system, Beijing Zhongshan Biotechnology, Inc.). The samples were immersed in PBS containing 3% H_2O_2 at 25 °C for 10 min to block nonspecific reactions. Subsequently, the sections were incubated in anti-collagen type II antibody working dilution at 4 °C overnight. After washing them with PBS three times, the samples were incubated in anti-rabbit IgG antibody working dilution at 37 °C for 20–30 min. The samples were incubated in 3,3’-diaminobenzidine tetrahydrochloride solution (0.5 mg ml^-1, with 0.01% H_2O_2 and Tris–HCl buffer solution as solvents, pH 7.6) after the anti-rabbit IgG antibody working dilution was removed by PBS. Then the samples were incubated in hematoxylin solution. Finally, collagen type II was stained brown and the basophilic mucus and proteoglycan around the chondrocytes were stained purple.

2.7. Statistical analysis

Data from all studies were analyzed using an analysis of variance. Results are reported as mean ± standard deviation. The significance level was set as p < 0.05.

3. Results

3.1. General description of the scaffolds

The porous PLLA scaffolds were fabricated by casting 0.1 g ml^-1 PLLA/1,4-dioxane solution into the gelatin particle assembly, followed by freeze-drying under reduced pressure and porogen leaching in water. The scaffolds resembled the macroscopic contours of the porogen assembly and had a pore size of 280–450 μm with good interconnectivity between pores. A large number of smaller pores of size 10–30 μm were also found in the skeletons, produced by a mechanism of thermally induced phase separation (TIPS). The apparent density of the scaffold was approximately 0.065 g cm^-3, and porosity was over 94% [16]. The remained content of gelatin was 117.6 mg g^-1 scaffold, measured by a spectrophotometrical method [16,32].

3.2. Weight loss and molecular weight change

The scaffold lost its weight rapidly during the initial stage, before the rate of weight loss slowed (Fig. 1a). After degradation for 39 weeks, 30% of the original weight had been lost. Meanwhile, the weight-average molecular weight (M_w) of the scaffold decreased almost linearly along with the degradation time (Fig. 1b). After 39 weeks, the M_w decreased to 80,000. The half-life of the scaffold in PBS is about 33 weeks according to Fig. 1b. It should be mentioned that the M_w (177,000) of the initial scaffold also decreased in comparison with its raw material (212,000),
implying the occurrence of degradation as a result of particle leaching in water at high temperature (70 °C).

3.3. Water uptake and thermal properties

The water uptake of the scaffold decreased initially along with the degradation time, then increased slightly (Fig. 2). The lowest value appeared at 9–12 weeks. The differential scanning calorimetry (DSC) thermograms of the PLLA scaffolds after degradation for different time are shown in Fig. 3. No significant difference in $T_g$ and $T_m$ can be found from Fig. 3 and the data listed in Table 1, although the $T_m$ seems to show a decreasing tendency (largest difference 1.3 °C). The data in Table 1 do show, however, that the melting enthalpy and the crystallinities ($X_c$) of the PLLA scaffolds were apparently increased along with the degradation time. At the longest time measured (39 weeks), the $X_c$ was increased by approximately 30% (from 17% to 21.9%).

3.4. Scaffold morphology

All the scaffolds maintained their porous microstructure and good interconnectivity regardless of the evolution of the degradation degree (Fig. 4). However, compared with the control scaffold (Fig. 4a), microcracks in the polymer skeletons (as indicated by the arrows) were observed after degradation for 9 weeks (Fig. 4b). Morphological change due to degradation could be obviously identified by SEM since 23 weeks. As shown in Fig. 4c–e, more cracks and even big holes (indicated by the arrows) could be found in these scaffolds.

3.5. In vivo histological investigation

Before implantation, the distribution of the chondrocytes was observed by CLSM, as shown in Fig. 5a, where

![Fig. 2. Change of water uptake of PLLA scaffold along with the in vitro degradation time.](image)

![Fig. 1. Weight loss (a) and weight average molecular weight (b) of PLLA porous scaffolds during degradation in PBS.](image)

![Fig. 3. DSC thermograms of the PLLA scaffolds after degradation for different time in PBS at 37 °C.](image)

<table>
<thead>
<tr>
<th>Degradation time (weeks)</th>
<th>$T_g$ (°C)</th>
<th>$T_m$ (°C)</th>
<th>$\Delta H_m$ (J g$^{-1}$)</th>
<th>$X_c$ (%)</th>
</tr>
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<tr>
<td>0</td>
<td>68.9</td>
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<td>34.7 ± 0.2</td>
<td>17.0 ± 0.1</td>
</tr>
<tr>
<td>9</td>
<td>72.3</td>
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<td>38.3 ± 0.2</td>
<td>18.8 ± 0.1</td>
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<tr>
<td>16</td>
<td>70.9</td>
<td>170.4</td>
<td>39.4 ± 0.4</td>
<td>19.4 ± 0.2</td>
</tr>
<tr>
<td>23</td>
<td>70.6</td>
<td>170.3</td>
<td>42.5 ± 0.6</td>
<td>20.9 ± 0.3</td>
</tr>
<tr>
<td>30</td>
<td>67.8</td>
<td>169.8</td>
<td>43.7 ± 0.3</td>
<td>21.5 ± 0.2</td>
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<tr>
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<td>68.4</td>
<td>169.6</td>
<td>44.6 ± 0.5</td>
<td>21.9 ± 0.3</td>
</tr>
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a large number of viable cells could be found within the scaffold. The cell density in the scaffold determined by DNA content was about $1 \times 10^6 \text{cm}^{-3}$. After implantation in the dorsum of athymic nude mice for 30–120 days, the scaffold seeded with chondrocytes were harvested. The implants were surrounded by thin vascularized capsules and were easily dissected from the subcutaneous tissue. No evidence of superficial infection or fistula formation was demonstrated in the experimental mice (Fig. 5 d and e). The scaffolds retained their original rectangular shapes after 30 days of implantation, but the shapes of the samples changed and distorted after 120 days. Histological examination of the implants using HE staining showed that the chondrocytes in their natural round morphology were surrounded by the newly formed matrices (Fig. 6a). Some small gaps (denoted by “P”) could be seen in the pictures, as indicated by the arrows. These gaps represent the traces of the polymer scaffold, which was removed by xylene during the process of HE staining. In the section of the implant after 30 days of culture in vivo, the gaps (P) were easy to find, implying that most of the scaffold still remained and occupied some spaces in the construct. However, in the

Fig. 4. SEM micrographs of cross-sections of the PLLA scaffolds after degradation for different times in PBS: (a) 0 weeks, (b) 9 weeks, (c) 23 weeks, (d) 30 weeks and (e) 39 weeks; (f) and (g) are higher magnifications of (d) and (e), respectively.
Fig. 5. (a) CLSM image to show the distribution of chondrocytes within the PLLA scaffold. Photographs of a nude mouse (b) after implantation of the chondrocytes/scaffold constructs and (c) after a 120 day follow-up. Gross view of the chondrocytes/scaffold constructs after in vivo cultured for (d) 30 days and (e) 120 days (left and right are the specimens obtained in the same culture condition).

Fig. 6. Hematoxylin and eosin staining of implants after implantation for (a) 30 days and (b) 120 days. The chondrocytes/scaffold constructs after in vivo culture for 120 days: (c) immunohistochemical staining of collagen type II, and (d) Alcian blue staining of GAG. Original magnification 100x. (For interpretation of the references in color in this figure legend, the reader is referred to the web version of this article.)
section of the implant after 120 days of culture (Fig. 6b), the number of gaps (P) had decreased significantly, implying that more of the scaffold had disappeared because of polymer degradation. Another important phenomenon is that some regions without cells (denoted as “C”) appeared. These regions were possibly initially occupied by some parts of the PLLA scaffold. Following their degradation, the secreted products of the chondrocytes filled up the new vacancies. Immunochemical staining (Fig. 6c) shows that the collagen type II content in these areas (C) is different from those matrices surrounding the chondrocytes. In these areas (C) the abundant collagen type II was stained brown; the lack of purple color (hematoxylin staining) reveals the absence of basophilic mucus and proteoglycan (Fig. 6c). Alcian blue staining further supports this conclusion (Fig. 6d).

4. Discussion

Porous PLLA scaffolds with an interconnected pore network have been successfully fabricated by using a porogen leaching and solvent removal technique. The apparent density of the scaffolds is about 0.065 g cm\(^{-3}\), and porosity is above 94% [16]. In this research, the degradation behavior of PLLA scaffolds was investigated over a 39 week period in PBS, and the growth of chondrocytes implanted in vivo was shown by histological sections.

In the process of leaching gelatin particles, the remaining gelatin adhered to the pore surfaces, which modified the PLLA scaffolds in situ by the so-called “physical entrapment” [33,34]. This effect endows the scaffolds with a good ability for cell infiltration and proliferation, thus avoiding the necessary surface modification step for this kind of biomaterial [15,28,35]. Another important feature is that the polyester should be degraded at a suitable rate, which is a rate comparable to tissue regeneration.

In general, there are four steps for degradation of the PLLA scaffold: (1) swelling and hydration of the polymer; (2) breakage of the ester bonds; (3) diffusion of the soluble degradation products; and (4) disappearance of the polymer scaffold chips [36]. The \(M_w\) of PLLA was already significantly decreased during the scaffold fabrication as a result of treatment in hot water. It decreased further as a function of incubation time in PBS (Fig. 1b). At 39 weeks, the \(M_w\) reached 80,000 (1/2 of that of the initial scaffold), while only 30% weight loss was measured. PLLA is a hydrolyzable polymer that follows a bulk degradation mechanism [37,38]. The ester bonds in the backbone chain break randomly to generate carboxyl groups, which in turn autocatalytically accelerate the degradation reaction, resulting in an exponential decrease of the molecular weight of a bulk PLLA material with degradation time. The intermediate degradation products are trapped inside the material before their molecular weights decrease to a critical value of about 1100 to be soluble in water [39]. However, in the present case, instead of an exponential regime, the \(M_w\) of the PLLA porous scaffold decreased in a linear manner. This inconsistency should be caused by the different microstructures of the materials. The scaffold is highly porous, with ultrathin wall thickness and interconnected pores throughout the entire structure. Hence, the generated acid is hardly accumulated within the walls to create a low pH environment, due to the neutralization of the buffer solution (pH 7.4), which wets almost all the surfaces of the pores.

The degradation of semicrystalline polymers begins mostly from the amorphous regions, where the segments of the macromolecules pack more loosely and can be more easily attacked by water molecules. Consequently, the amorphous regions are preferentially removed, leading to the relative increase of the crystallinity of the remaining material. On the other hand, the breakage of the segments leads to a decrease in molecular entanglement and an increase in chain mobility. The chains could then reorganize themselves to a more orderly macromolecular arrangement. Therefore, the crystallinity of the PLLA scaffolds after degradation is apparently increased (Fig. 3 and Table 1). This phenomenon is also reported in the in vitro degradation of biodegradable sutures [40]. Although the crystallinity is improved, the crystallites may become more disordered. A smaller molecular weight is of benefit to molecular movement too. Hence, the melting range of the degraded PLLA scaffolds shifted to lower temperatures [41].

As mentioned previously, degradation in the amorphous regions leads to the breakage of the molecular chains connecting between the crystalline domains, resulting in defects of scaffolds in PBS [40]. In spite of the neutralization effect of the buffer, the concentration of the carboxylic groups produced by degradation is higher to some degree in the scaffold interior, leading to faster wall degradation and therefore big holes in the scaffolds (Fig. 4d and e).

The relatively high water uptake ratio at the initial stage is caused by the water affinity of the remained gelatin (117.6 mg g\(^{-1}\) scaffold) [16]. Along with the continuous dissolution of the remained gelatin, the water uptake is decreased until 9 weeks. The slight increase of the water uptake at this later stage is attributed to the relatively larger amount of hydrophilic groups, i.e. carboxyl and hydroxyl groups. This alteration is consistent with the scaffold feature and the results of weight loss.

The microenvironment is an important factor influencing the performance of a scaffold. Previous research has revealed that some enzymes (such as proteinase K and lipase PS) can modulate the degradation of PLLA in vivo, especially for the release of degradation products [42]. On the other hand, tissue engineering requires that the degradation rate of the polymer scaffold should match the regeneration of new tissues. Hence, the relationship between new tissue formation and scaffold degradation in vivo should be studied carefully. To observe the degradation of the PLLA scaffold during new tissue formation, the chondrocytes were seeded into the scaffold, and the cells/scaffold construct was then implanted subcutaneously in the dorsum of athymic nude mice. The cell–material
constructs were harvested after in vivo cultivation for 30–120 days. Because of the gelatin remaining in the scaffold, the chondrocytes showed good biological performance. The chondrocytes were found inside the scaffold surrounded by a mass of matrix secreted by the cells. From a wider viewpoint, the newly formed tissue and the scaffold had fused together and were not easily distinguished.

The scaffolds maintained their original rectangular shapes after 30 days of implantation. However, during long-term implantation the scaffold was continually degraded, leading to a weakening of the mechanical strength of the constructs. Consequently, the shapes of the samples were changed and distorted. Since the samples were not fixed in the murine subcutaneous tissue, they were subject to the influence of external compression forces, and thus their shapes were changed. Although the tissue engineering approach is a potential alternative for cartilage reconstruction, there are still many unresolved issues. The loss of the precise shape of the tissue-engineered constructs is a common problem that has been recognized by other researchers [43]. Other reasons include inadequate cell seeding, faster degradation rate of the scaffold and insufficient mechanical strength.

Histological examination of the constructs implanted for 30–120 days was performed to assess the degradation of the scaffold and the response of the cultured cells (Fig. 6). The traces of polymer scaffold (P) in the section of the implant after 30 days of culture suggest the existence of a large amount of PLLA. However, after 120 days of culture in vivo, most of the polymer scaffold (P) had disappeared. Some regions with no chondrocytes but only collagen (C) could be clearly identified (Fig. 6b–d), as indicated by the arrows. Without chondrocytes and GAGs, such as chondroitin sulfate and hyaluronic acid, the matrices in these regions cannot form a cross-linked network like natural cartilage, and this may produce mechanical defects in the newly formed constructs. This phenomenon is very similar to the results of Furukawa et al. in cartilage resurfacing experimental defects in the rabbit knee [44]. They found that the amount of collagen type II in the self-repaired cartilage after 12 weeks was higher than in normal cartilage but the hexosamine was lower, indicating that the cartilage fibrosis was caused mainly by the lack of proteoglycan. According to our results, a still slower degradation rate and stronger mechanical strength throughout most of the period of cartilage regeneration would be more promising. Shieh and Vacanti [43] drew the same conclusion when they studied the cartilage restoration behavior in vivo, using polyglycolic acid, poly-$\varepsilon$-caprolactone and poly-4-hydroxybutyrate meshes as the scaffolds.

Compared with the degradation in vitro, the degradation rate of the PLLA scaffolds in vivo was much faster. While most parts of the scaffold existed and maintained intact pore structure even after 23 weeks of degradation in vitro (Fig. 4b), it is hard to find the scaffold in the sectioned sample after in vivo implantation for 120 days (17 weeks) (Fig. 6b and c). The environment in vivo, including the ease of substance diffusion and the existence of enzymes, is usually regarded as accelerating the degradation of the polymer scaffold. Therefore, to optimize the materials for tissue engineering, it is not enough to observe scaffold degradation behavior only in vitro; instead, experiments in vivo to determine the relationship between the scaffold disappearance and the new tissue restoration are necessary.

5. Conclusions

The porous PLLA scaffolds degrade in PBS (pH 7.4) at 37 °C in vitro at a relatively slow rate. Different from the exponential degradation of bulk material, we find here that the molecular weight decreases linearly as a function of degradation time. The highly porous structure with interconnected pores ensures sufficient wetting of the scaffold by the buffer solution, and greatly reduces the accumulation of generated acids within the pore walls. This will surely eliminate the autocatalytic effect brought about by the acids, which would mean that the “burst” disappearance of the bulk PLLA material may not happen for the porous scaffold, or at least would not be so severe. Nonetheless, due to the poorer exchanging ability of the scaffold interior, bigger defects are formed there. The degradation rate is accelerated in vivo. After 120 days of culture subcutaneously in nude mice, most of the scaffold has disappeared. The regions initially occupied by the polymer scaffold are filled with collagen type II, with no evident basophilic proteoglycan. The scaffold is also unable to maintain its predesigned shape after a long period of implantation, due to the weakening of the mechanical strength of the construct. In order to regenerate cartilage with ideal properties, we suggest that a scaffold is needed which maintains enough mechanical strength throughout most of the tissue regeneration process. Moreover, the in vivo study of a scaffold seeded with targeted cells will draw more accurate conclusions in terms of scaffold degradation. This is an indispensable step towards applications of any scaffolds for tissue engineering and regenerative medicine.

Acknowledgements

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