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Degradation of three dimensional poly(l-lactic acid) scaffolds modified by gelatin

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In vitro degradation of poly(l-lactic acid) (PLLA) scaffolds modified by gelatin was carried out in 0.01 M NaOH solution at 37°C for 5–6 days. The mass loss, pH value, viscosity-average molecular weight, morphology and thermal behavior during degradation were studied. The results showed that the prepared scaffolds were interpenetrating porous structure and the increased degradation time; both the viscous-average molecular weight and melting peak showed a clear upward trend, while the crystallinity and mass loss exhibited a downward trend. The semi-logarithmic linear relationship between the viscosity-average molecular weight and degradation time indicates an autocatalytic process. The mass of the PLLA scaffolds decreased by 10.33 % after 2 days of degradation and decreased by 86.19 % to the end of the experimental period. The decrease in the viscous-average molecular weight and a relatively little mass loss in the beginning of the degradation period indicate the bulk degradation. The gradual mass loss is indicated not only by the bulk degradation mechanism but also a surface erosion mechanism. The results obtained by the *in vitro* degradation in a NaOH solution at 37°C can find useful applications in solving the biocompatibility of PLLA scaffolds.

Keywords:Poly(l-lactic acid), scaffolds, degradation, tissue engineering, gelatin.

Разложение *in vitro* каркасов поли(1-молочной кислоты) (PLLA), модифицированных желатином, проводили в 0.01 М растворе NaOH при 37°C в течение 5–6 дней. Исследована потеря массы, значение pH, средневязкостная молекулярная масса, морфология и термическое поведение при разложении. Результаты показали, что синтезированные каркасы имели взаимопроникающую пористую структуру и повышенное время разложения. Средневязкостная молекулярная масса, как и пик плавления демонстрировали четкую тенденцию к росту, при этом кристалличность и потеря массы снижались. Полулогарифмическая линейная зависимость между средневязкостной молекулярной массой и временем разложения указывает на автокаталитический процесс. Масса каркасов PLLA уменьшилась на 10.33 % после деградации в течение 2 дней и на 86.19 % в конце экспериментального периода. Снижение средней вязкой молекулярной массы и относительно небольшая потеря массы в начале периода деградации подтвердили объемную деградацию. На постепенную потерю массы указывает не только механизм объемной деградации, но и механизм поверхностной эрозии. Результаты, полученные разложением *in vitro* в растворе NaOH при 37°C, могут найти полезное применение для решения проблемы биосовместимости каркасов PLLA.

Деградація тривимірних каркасів полі(1-молочної кислоти), модифікованих желатином. *Ye Zhang, Hong-ming Liu.*

Розкладання *in vitro* каркасів полі(1-молочної кислоти) (PLLA), що модифіковані желатином, проводили у 0.01 М розчині NaOH при 37°C протягом 5–6 днів. Досліджено втрату маси, значення pH, середньов'язкісну молекулярну масу, морфологію і термічну поведінку під час розкладання. Показано, що синтезовані каркаси мають

взаємнопроникну пористу структуру і підвищений час розкладання. Средньов'язкісна молекулярна масса, як і пік плавлення демонстрували чітку тенденцію до зростання, при цьому кристалічність і втрата маси показали тенденцію до зниження. Напівлогарифмічна лінійна залежність між середньов'язкісною молекулярною масою і часом розкладання вказує на автокаталітичний процес. Маса каркасів PLLA зменшилася на 10.33 % після деградації протягом 2 днів і на 86.19 % в кінці експериментального періоду. Зниження середньов'язкісної молекулярної маси і відносно невелика втрата маси на початку періоду деградації підтвердили об'ємну деградацію. На поступову втрату маси вказує не тільки механізм об'ємної деградації, а й механізм поверхневої ерозії. Результати, отримані розкладанням *in vitro* у розчині NaOH при 37°C, можуть знайти корисне застосування для вирішення проблеми біосумісності каркасів PLLA.

1. Introduction

Development of tissue engineering has accelerated the demand for biodegradable and biocompatible materials. Poly(L-lactic acid) (PLLA) has been widely used as a biomaterial because its degradation products are biocompatible, biodegradable and recyclable. PLLA has therefore gained the approval of US Food and Drug Administration (FDA) for a variety of human clinical applications [1]. Although synthetic materials were widely used, the lack of tissue compatibility and resistance to biological environment were the problems that remain to be resolved [2]. Recently, PLLA blends with different natural and synthetic polymers have been explored for biomedical applications such as drug delivery, implants, sutures, and tissue engineering [3–6].

The biodegradation behavior of PLLA, being an important property determining its final applications, was influenced by geometrical shape, size, crystallinity, pH value of the medium, temperature, irradiation, and so on [7]. Many previous studies on the degradation of PLLA involved specimens in the form of films, foams, rods, or plates and so on [8–10].

It is known that surface modifications of the biodegradable polymers in alkaline solution could be used to generate a hydrophilic and rough surface for cell attachment [11]. Thus, this study investigated the detailed degradation of three-dimensional PLLA scaffolds modified by gelatin in the alkaline solution. Characterization and discussions on the mass loss, viscosity-average molecular weight, morphology and the thermal behavior of the PLLA scaffolds modified by gelatin are investigated.

2. Experimental

Poly(l-lactic acid) (PLLA, Mn = 4.32·104 g/mol) was kindly donated by Prof. Xue-Si Chen, Changchun Institute of Applied Chemistry Chinese Academy of Sciences. Gelatin was purchased from Sigma-Aldrich Co. (St Louis, USA). Tetrahydrofuran used was of analytical reagent grade.

PLLA scaffolds modified by gelatin were produced in our laboratory by phase separation, solvent replacement and freeze-drying [12]. 21 weighed scaffolds were completely immersed in the 0.01 M NaOH solution at 37°C for the degradation test according to the 100 mg dissolved in 50 ml degradation medium. Three of the scaffolds were taken as a group. The NaOH solution was replaced once a day. After a certain time, one group of the scaffolds was removed from the medium, washed with water thoroughly and dried in a vacuum oven at 45°C for 2 h. The mass losses of the PLLA scaffolds before and after degradation were tested.

The molecular weight was measured by the viscosity method in a dilute polymer/chloroform solution (0.1 g/dL) using an Ubbelohde viscometer (Type 0c) at 25°C. The viscosity-average molecular weight was calculated by the following equation [17]:

$$[\eta] = 5.45 \cdot 10^{-4} \cdot M_{\eta}^{0.73}, \quad (1)$$

$$\eta_r = \frac{t}{t_0}, \quad (2)$$

$$\eta_{sp} = \frac{\eta - \eta_0}{\eta_0} = \eta_r - 1, \quad (3)$$

$$[\eta] = \frac{2(\eta_{sp} - \ln\eta_r)^{1/2}}{C}. \quad (4)$$

Here η_r is the relative viscosity, which is the ratio of absolute viscosity of the solution to that of the solvent at the same temperature; t and t_0 are the times required for the solution and solvent to flow through the same capillary at the same height, respectively; $\eta_{sp}$is the specific viscosity, which is defined as the percentage of incensement in solvent viscosity caused by the addition of a high polymer solute; C is the solution concentration.

Morphology of the PLLA scaffolds modified by gelatin was recorded on a Philips

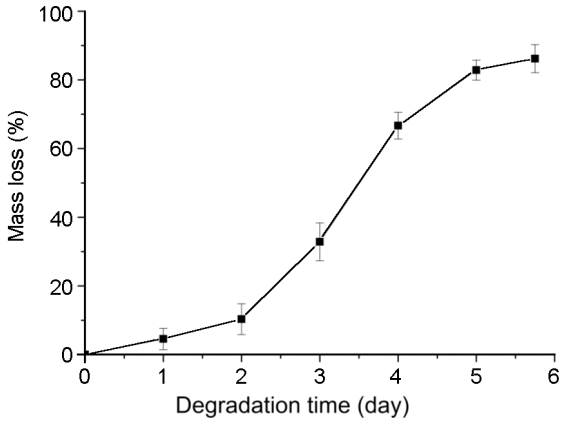

Fig. 1. The mass loss of PLLA scaffolds after different periods of degradation.

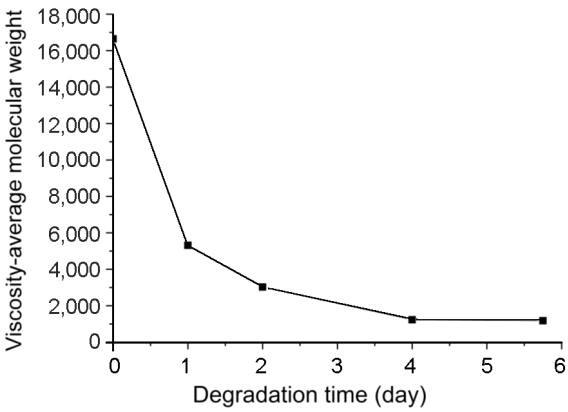

Fig. 2. Changes of viscous-average molecular weight of PLLA scaffolds after different periods of degradation.

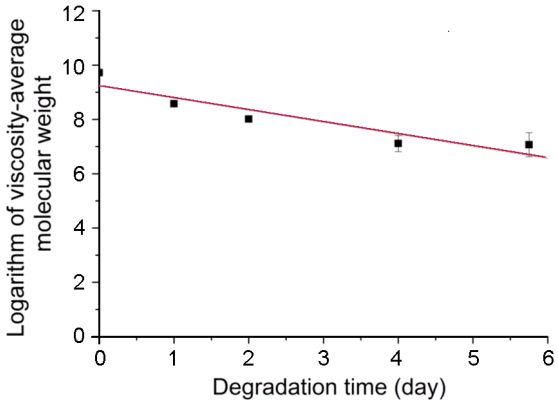

Fig. 3. The semi-logarithmic linear relationship between viscosity-average molecular weight and degradation time.

XL30 scanning electron microscope (SEM) after gold coating.

DSCs were measured using a Perkin-Elmer DSC 7 (Perkin-Elmer, Norwalk, CT, USA). 5–10 mg of Tet-loading PLLA scaffolds were sealed in aluminum pans and heated at 10°C/min from 10°C to 240°C and under N_2 flow (100 mL/min).

3. Results and discussion

As shown in Fig. 1, the mass losses of the PLLA scaffolds were measured throughout the degradation period. The PLLA scaffolds exhibited a little mass loss in the first 2 days of the degradation and a gradually increased mass loss after 2 days. During the degradation, the pH value of the NaOH solution dropped slightly from 11.7 to 11.6 after 1 day of the degradation, and this decrease was not observed afterwards.

As shown in Fig. 2, the viscous-average molecular weight of the PLLA scaffolds decreased sharply in the first 2 days, and then decreased gradually in the NaOH solution. Additionally, the viscosity-average molecular weight of the PLLA scaffolds dropped in a semi-logarithmic linear manner with degradation time Fig. 3. Obviously, the degradation of the PLLA scaffolds was influenced by autocatalytic process [13]. The results were the same as the decrease in the viscosity-average molecular weight of PLLA fibres after in vitro degradation in a dilute alkaline solution and phosphate buffered saline at 37°C [14]. After 5.75 days of the degradation, the viscous-average molecular weight of the scaffolds decreased significantly from 16644 to 1174.

In this paper, the decrease in the viscous-average molecular weight of the PLLA scaffolds suggested that the PLLA scaffolds were suffered from bulk degradation. The bulk degradation mechanism could also be confirmed by a relatively little mass loss in the beginning degradation period. Then, the gradual mass loss indicated that the degradation of the PLLA scaffolds was caused not only by the bulk degradation but also by surface erosion.

SEM micrographs of the PLLA scaffolds with degradation time were shown in Fig. 4. The PLLA scaffolds had a smooth surface before the degradation Fig. 4a. After immersing in the NaOH solution for 30 min, rough surfaces with some micro-cracks were observed. With the prolongation of degradation time, more cracks appeared and they were larger Fig. 4b–Fig. 4f. It was apparent that the pore size is small and uniform before the degradation. After the degradation, the pore size increased significantly. After 5.75 days of the degradation, the overall shape of pores can not be seen, and an in-

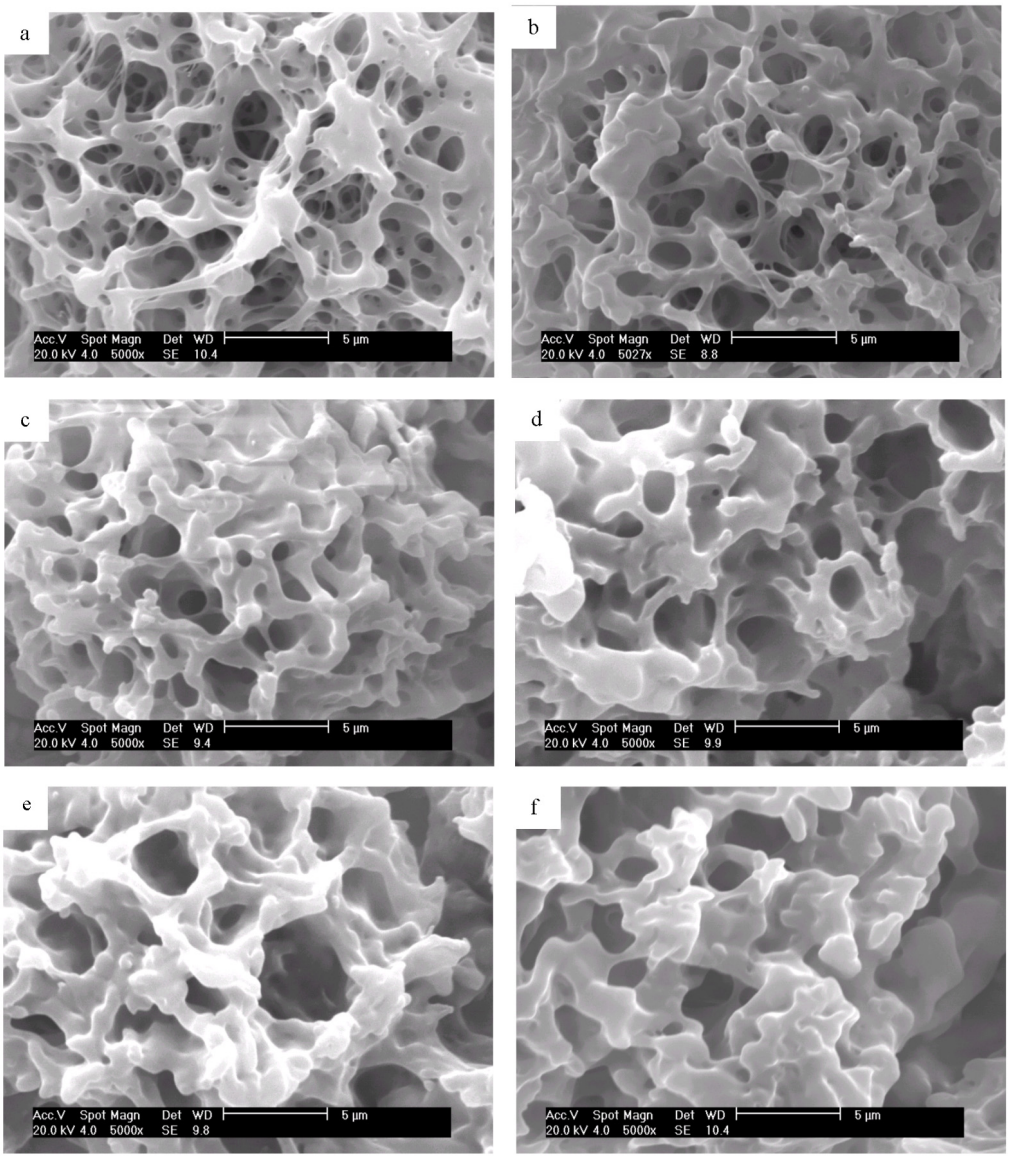

Fig. 4. SEM micrographs of PLLA scaffolds before (a) and after 1 day (b), 2 days (c), 4 days (d), 5 days (e), and 6 days (f) of degradation in NaOH solution.

terpenetrating porous structure was appeared Fig. 4f.

Fig. 5 shows DSC thermograms of the PLLA scaffolds before and after degradation in the NaOH solution. The curves exhibit little changes in their shapes. There exists only one melting peak in the first 3 days and double-melting peaks after 4 days of the degradation. The double-melting behavior of the PLLA has been explained by a melt-recrystallization model [15–17]. The low-temperature melting peak in the DSC thermograms is attributed to the melting of the primary crystallites formed at the crystallization temperature, and the high-temperature melting peak reflects the stacks of relatively perfect thicker lamella resulting from recrystallization during the heating scan. On the other hand, the melting behavior of semi-crystalline polymers is affected by the melting of the primary crystallites, recrystallization and the melting of re-crystallites.

The melting temperature (T_m) indicated by the maximum values of the peak shifted slightly to lower temperatures with degradation time. This decrease in the melting temperature of the PLLA scaffolds is clearly shown in Table. However, the crystallinity of the PLLA scaffolds (X_c) increased significantly from 43.18 % to 49.37 % in the first day of the degradation. Then, the crystallinity increased slightly from 49.37 % to 50.58 %.

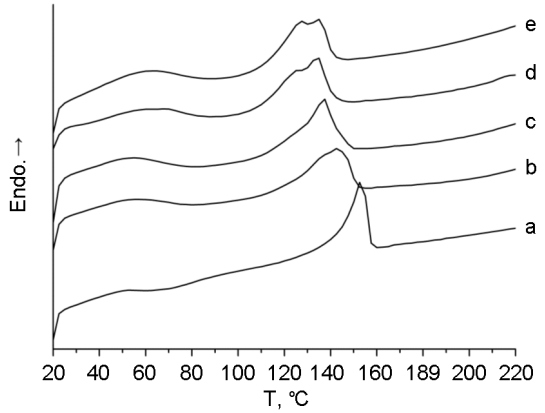

Fig. 5. DSC thermograms of PLLA scaffolds before (a) and after 1 day (b), 2 days (c), 3 days (d), and 5.75 days (e) of degradation in NaOH solution.

Degradation of the PLLA scaffolds begins mostly in the amorphous regions where the segments of the macromolecules are packed more loosely and could be more easily attacked by water. The breakage of the segments resulted in the decrease of the molecular entanglement and the increase of mobility of the macromolecular chains. The chains could then re-arrange themselves more orderly. So the crystallinity of the PLLA scaffolds after the degradation apparently increased. Obviously, the perfection of the crystallites in the PLLA scaffolds is deteriorated by the above process. Moreover, the mobility of the macromolecular chains was promoted by the decrease of the molecular weight. Thus, the melting temperature of the degraded PLLA scaffolds decreased notably. These results are consistent with that of PLLA fibres after degradation in PBS [14].

4. Conclusion

Degradation of the PLLA scaffolds modified by gelatin is characterized by autocatalytic phenomena, bulk degradation and surface erosion. The viscosity-average molecular weight of the PLLA scaffolds dropped in a linear semi-logarithmic manner and decreased by 92.95 % after 5.75 days of the degradation. The mass of the PLLA scaffolds modified by gelatin was decreased slightly at the beginning and then decreased gradually. The crystallinities apparently increased slightly. The surface of the PLLA scaffolds became rough, gave rise to microcracks. Then, the PLLA scaffolds became fragile. The pore size increased significantly.

Table. T_m, ΔH_m (the melting enthalpy of the sample) and X_c of PLLA scaffolds modified by gelatin after degradation in NaOH solution

Degradation time, day	T_m, °	ΔH_m, J/g	X_c, %
0	153.6	40.16	43.18
1	146	45.91	49.37
2	138.5	46.15	49.62
3	136.2	47.04	50.58
5.75	127.5	47.04	50.58

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