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Synthesis and application of PLGA labeled with ^{125}I

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Abstract The weight loss in vivo degradation of poly (lactide-co-glycolide) (PLGA) radiolabeled with ^{125}I was investigated. PLGA with molecular weight (M_w) of 84000(LA/GA = 85/15) were labeled with ^{125}I in the chloroform media by circularly heating and round films of about 15 mm in diameter were formed. The composition and M_w of the ^{125}I-PLGA were characterized by ^{1}H-NMR and viscosimeter. The weight loss of this copolymer in vitro and in vivo degradation was quantified by determining radioactivity of materials. The results indicated that PLGA exhibited significantly faster degradation in vivo than that of in vitro conditions.

Key words Poly (lactide-co-glycolide), ^{125}I labeling, Degradation.

CLC number Q819

1 Introduction

Poly(lactide-co-glycolide) (PLGA), a co-polymer made from lactide (LA) and glycolide (GA), is a kind of bio-degradable macromolecular material, and has been widely used in tissue engineering, controllable drug release system and orthopaedics [1-3]. Degradation properties are of crucial importance in the biocompatibility of PLGA. The rate of degradation may affect many cellular and tissue processes including cell growth, tissue regeneration, and host response [4]. Hence, the evaluations and investigations of degradation properties and process of PLGA are very important and valuable. Though weight loss is an important parameter of degradation properties of PLGA, reports on weight loss of PLGA in vivo degradation are still rare.

Under the tissue reaction after the implantation of PLGA, the implant would be surrounded by fibrous tissues, which would even grow into the materials' structure during the degradation process [5, 6]. Hence, it is difficult to separate the PLGA and measure the weight directly. At present the morphometric analysis and radiolabeling technique are in common use in the research field of vivo degradation. However, the reliability and veracity of morphometric analysis is low because many factors would influence the results [7, 8], and the cost and risk of radioactive pollution of radiolabeling with ^{3}H and ^{14}C are high [9]. The radiolabeling technique with ^{125}I was reported to research distribution and pharmacokinetics of co-polymers such as PLA-PEG and PLA-PEO [10, 11]. According to these reports, the co-polymers contain an aromatic moiety for radiolabeling and M_w of the materials is lower than 20000.

The purpose of this study was to prepare a high molecular weight PLGA radio-labeled with ^{125}I, and then characterize the composition and M_w of the ^{125}I-PLGA by ^{1}H-NMR and viscosimeter. Subsequently, we reported the rate of weight loss in vitro and in vivo degradation of this co-polymer.

2 Experiments and methods

2.1 Agents and instruments

SN-682 γ counter (Rihuan Instrument Factory,

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Shanghai), JNMR DMX500 spectrometer (Joel®, Tokyo, Japan), M－1120 Electronic Balance (Satoris®, Germany), Chloroform ($CHCl_3$) (Shanghai Zhengxin Chemical Plant, China), $Na^{125}I$ (Chengdu Gaotong Isotope Corporation, China Nuclear Group).

2.2 Radiolabeling with ^{125}I

Poly(lactide-co-glycolide)(PLGA) was provided by Faculty of Macromolecule of Fudan University. The ratio of LA and GA was 70 to 30, and the M_w was 84000. 1.0g PLGA was dissolved in 100 mL chloroform and 5μL $Na^{125}I$ (11MBq/μL) was added. The solution was stirred for 2 min, then tightly capped and the reaction was conducted by being heated circularly between 25℃ and 110℃ for 2 h. The reaction was stopped by addition of sodium meta-bi-sulfite and stirred for another 2 min. Subsequent to displacing any free (unbound) [^{125}I] iodide with 1 mg of potassium iodide, the solution was extracted by using distilled water at least three times, until no free radioactive could be detected in distilled water. Then the radioactivity preparation solution was separated by centrifuge at 1500 r·min^{-1} and stored at −20℃ prior to use.

50 μL labeled sample solution was applied to GF silica gel divided into 0.5 cm strips. The sample was spread by mixture of chloroform and ethanol (1:1) for 20 min. Then the gel was dried, the radioactivity of each strip assayed, and the radiochemical purity calculated.

0.5 mL, 1 mL and 1.5 mL radio-labeled preparation solution, respectively, was volatilized in a disk about 15 mm in diameter for 24h, then the ^{125}I-PLGA was allowed to solidify and form round films of about 15 mm in diameter. Then, the radioactivity of ^{125}I-PLGA with weights of 5 mg, 10 mg and 15 mg, respectively, was measured by the SN－682 γ counter, and the specific radioactivity (min^{-1}·mg^{-1}) was calculated.

Composition of ^{125}I-PLGA was determined using ^1H-NMR spectroscope at 500 MHz in $CDCl_3$ solutions containing tetramethylsilane (TMS) as reference at 25℃.The composition of the sample was calculated by comparing the LA single proton (δ= $(5.14-5.24)\times10^{-6}$) with the GA methylene group (δ $=(4.69-4.89)\times10^{-6}$) in PLGA [12].

2.3 In vitro degradation of ^{125}I-PLGA

Three samples of ^{125}I-PLGA and PLGA weighing 10mg were steeped in 10mL 0.1mol/L phosphate buffer (pH 7.4) in an incubator at 37°C according to ISO 10993.13-1998 [13], for defined time intervals, namely 7 d, 28 d, 56 d and 84 d, respectively. Then three samples were removed from the buffer successively, washed with distilled water and subsequently lyophilized. The weight and viscocity of the materials were measured and M_w was calculated using Mark-Houwink equation [14].

2.4 In vivo degradation of ^{125}I-PLGA

Prior to implantation, 12 SD rats were weighed and identified. The animals were anesthetized by intramuscular injection of ketamine hydrochloride and xylazine cocktail combined. Then the animals' hair on the back was thoroughly clipped with electric clippers. The surgical site was scrubbed with a germicide soap, wiped with 70% alcohol and painted with povidone iodide and draped. Veterinary ophthalmic ointment was applied to protect the corneas from excessive drying. A skin incision was made large enough to accommodate the test samples. Pockets were formed by blunt dissection. A 10 mg rounded ^{125}I-PLGA film was gently tiled into each pocket. The skin was closed using wound clips. At defined time intervals, namely 7 d, 28 d, 56 d and 84 d, respectively, three rats randomly selected were killed by injecting excessive ketamine. Then the materials with the tissue around (including derma and sub-derma tissue) were removed from the back together because the materials and the tissue couldn't be separated, and the radioactivity was measured by SN-682 γ counter. The weight change of ^{125}I-PLGA was calculated as:

$$M_c=C_1/(C_0\times e^{-0.69315t/T}) \quad (1)$$

where M_c is the weight of the materials (mg), C_1 is the radioactivity measured in defined time intervals (min^{-1}), C_0 is the radioactivity per unit weight of the materials measured at the beginning of the experiment, T=59.6d is the half-life of ^{125}I, and t is the time intervals between C_1 and C_0 (d).

2.5 Statistical analysis

Individual parameters were expressed as mean ± SD, and statistically evaluated by *t* test using SAS 6.12 software.

3 Results

3.1 Radio-labeling with ^{125}I

Table 1 shows the specific radioactivity of ^{125}I-PLGA. No significant difference is found between materials with different weights ($P>0.05$). The radiochemical purity (results not shown) of ^{125}I-PLGA was more than 98%.

Table 1 Radioactive counting of ^{125}I-PLGA with different weights ($n=3$)

Weight / mg	Radioactivity / min^{-1}	Specific radioactivity / min$^{-1}\cdot$mg^{-1}
5.0	302791 ± 1519	60438 ± 300
10.1	589060 ± 10730	58323 ± 1073
15.0	915115 ± 16598	61008 ± 1107

Fig. 1 and Fig. 2 represent the ^{1}H-NMR spectra of PLGA and ^{125}I-PLGA.

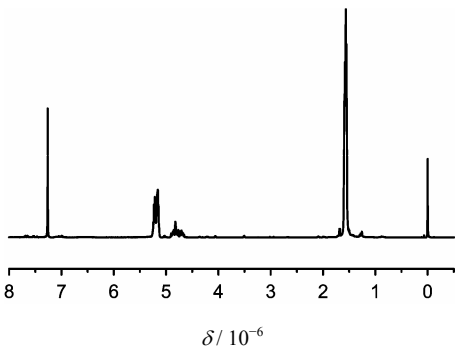

Fig.1 ^{1}H-NMR spectrum of PLGA.

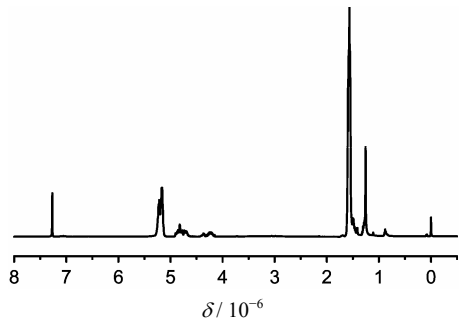

Fig.2 ^{1}H-NMR spectrum of ^{125}I-PLGA.

3.2 In vitro degradation of ^{125}I-PLGA

Fig. 3 and Fig. 4 indicate the weight loss and M_w change of 10mg ^{125}I-PLGA and PLGA in phosphate buffer.

There was no statistical difference of weight loss and M_w change between ^{125}I-PLGA and PLGA ($P>0.05$) in vitro condition.

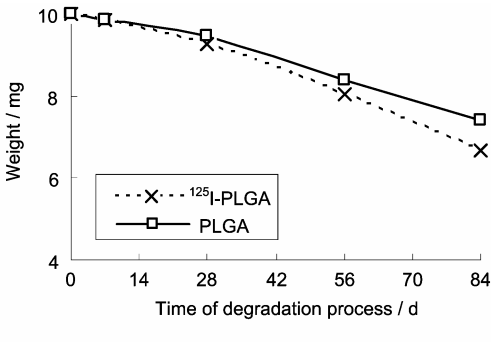

Fig.3 The weight loss of ^{125}I-PLGA and PLGA during in vitro degradation.

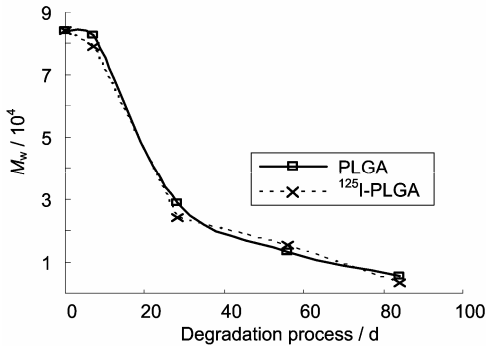

Fig.4 M_w change of ^{125}I-PLGA and PLGA in vitro degradation.

3.3 In vivo degradation of ^{125}I-PLGA

Table 2 shows the weight loss of 10mg ^{125}I-PLGA during degradation process in vivo.

Table 2 The weight loss (%) of ^{125}I-PLGA during degradation process in vivo

Time intervals / d	*n*	Radioactivity / min^{-1}	Disintegration rate	Weight loss / %
7	3	532251±23236	0.922	2
28	3	367177±44263	0.722	14
56	3	175956±11576	0.521	43
84	3	79735±3322	0.376	64

4 Discussion

4.1 PLGA radio-labeling with ^{125}I

This is the first report, to the best of our knowledge, on the preparation of high molecular weight PLGA labeled with ^{125}I. Compared with chloramine T method applied in ^{125}I-labeling of polymers in existence [10, 11], the labeling technique established in this study has the following advantages:. 1) We radiolabeled the PLGA without containing an aromatic moiety. 2) The ^{125}I is conjugated to the finished product of high M_w PLGA directly. 3) The cost and risk of radioactive pollution are low. The new synthesized ^{125}I-PLGA released γ rays (Table 1), and the radiolabeling efficiency was in accordance with the requirement of labeling technique.

The ^{1}H-NMR spectra of PLGA and ^{125}I-PLGA show that after the labeling process there is a small peak appearing at 4.3×10^{-6} in Fig. 2, and the ratio of area of peak at $(5.14\text{-}5.24)\times10^{-6}$ to area of peak at $(4.69\text{-}4.89)\times10^{-6}$ in Fig. 1 is larger than in Fig. 2. Since the peak at $(5.14\text{-}5.24)\times10^{-6}$ stands for the LA single proton (CH) and $(4.69\text{-}4.89)\times10^{-6}$ for the GA methylene group (CH_2) [12], it indicates that the ^{125}I conjugated to the PLGA by replacing the H on hypo-methyl of LA (see Fig.5) and the new peak at 4.3×10^{-6} is supposed to be obtained through the influence of the (-^{125}I) block.

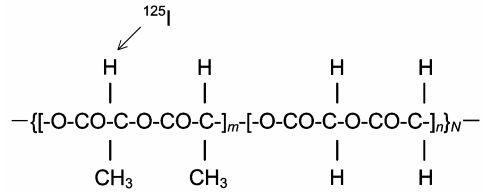

Fig.5 Replacement of H on hypo-methyl of LA in PLGA by ^{125}I.

4.2 In vitro degradation of ^{125}I-PLGA

Since there was no statistical difference of weight loss and M_w change between ^{125}I-PLGA and PLGA in vitro degradation, the radio-labeling with ^{125}I had negligible influence on the degradation of PLGA. Further, it was proved that the ^{125}I was labeled to PLGA with high M_w successfully. Hence, radio-labeling technique with ^{125}I could be applied to the research field of degradation process of PLGA.

4.3 In vivo degradation of ^{125}I-PLGA

Since the fibrous tissue would grow into the materials' structure during in vivo degradation [5, 6], it is difficult to measure the weight loss directly and quantitatively. However, the γ rays released from ^{125}I-PLGA can be detected by γ counter directly without separating the material from tissue. In this study, the weight loss of ^{125}I-PLGA in rats during degradation was determined by measuring radioactivity. The results (see Table 2) show that the PLGA exhibits much faster degradation in vivo than in vitro conditions. The results are, hence, similar to that of our previous study [15].

5 Conclusion

As noted above, there seem no previous reports on weight loss of PLGA in vivo degradation by preparing high molecular weight ^{125}I −PLGA. In this study we reported the ^{125}I conjugated to the PLGA by replacing the H on hypo-methyl of LA. The radiolabeling with ^{125}I had little influence on the degradation of PLGA. The weight loss of ^{125}I-PLGA in vivo measured by monitoring radioactivity indicates the faster degradation than that in vitro conditions. The radiolabeling technique established in this study will be helpful to the research of degradation behavior of other biodegradable polymers.

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