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Body distribution of poly(D,L-lactide-*co*-glycolide) copolymer degradation products in rats

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Abstract Poly (D,L-lactide-*co*-glycolide) (PLGA) copolymers are among the few synthetic polymers approved for human use, but the biocompatibility of PLGA-derived oligomers and particles remains questionable. Here, high molecular weight PLGA (Mw = 32,000) was radiolabeled with ^{125}I in chloroform solution, and the body distribution of PLGA copolymer degradation products was examined following subcutaneous implantation of round ^{125}I-PLGA films on the back of Sprague Dawley rats. Autoradiographic images of the PLGA implant taken at 2, 4, 6, 8, 10, and 12 weeks revealed that the central portion of the film degraded much more rapidly than the marginal portions. Examination of the body compartment distribution at these time points revealed that over one-half of the radioactivity was recovered from skin. The remaining radioactivity was concentrated in the blood, liver, and kidneys. Radioactivity steadily appeared in the blood and remained elevated up to 12 weeks after implantation, while the liver to kidney distribution began to decrease after 6 weeks. Cumulatively, these results indicate that the clearance of degraded particles and fragments from the implantation site is extremely delayed. Moreover, the degraded particles and fragments were selectively concentrated in the liver and kidneys, following release of degraded products into the bloodstream from the implantation site.

1 Introduction

Poly(D,L-lactide-*co*-glycolide) (PLGA) copolymers are among the few synthetic polymers approved for human use. Understanding and controlling the degradation of these biodegradable copolymers as well as the effects that the degradation products have on the body is crucial for long-term clinical success. Over the past few decades, previous studies have revealed that in vitro and in vivo PLGA degradation are the result of several processes occurring simultaneously. These include water uptake, swelling, ester hydrolysis, diffusion of oligomers and degradation products, and local pH drop [1–3].

The PLGA monomers, lactic acid and glycolic acid, are non-toxic and can be removed from the body by normal metabolic pathways [4]. However, the biocompatibility of degraded oligomers and particles remains questionable. For example, oligomers and polymer particles can elicit inflammatory responses, sometimes causing tissue necrosis. This has been documented in several long-term studies [5–9]. In addition, as diffused oligomer and particles continue to degrade, lactic and glycolic acid are released, resulting in a local drop in pH in poorly vascularized areas [10, 11].

The exposed tissue and the periods of retention are of crucial importance in the biological response to polymer particles and oligomers. Therefore, investigating the distribution and pharmacokinetics of PLGA degraded products is necessary for effective prediction of host responses to PLGA in particular applications. Kulkarni [12] first studied the metabolic pathways of PLA by a ^{14}C radiolabeling technique. However, the cost and risk of radial pollution associated with ^{14}C radiolabeling are high. As an alternative, the chloramine T method of radioiodinated low molecular weight block copolymer [13, 14] synthesis is now used to investigate the pharmacokinetics and biodistribution of copolymer particles. In the current study, the distribution and metabolism of ^{125}I-labeled high molecular weight PLGA (Mw = 3.2×10^4) which had been prepared via a new labeling technique, was investigated in laboratory rats.

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2 Materials and methods

2.1 Materials

PLGA, comprised of a lactic/glycolic molar ratio of 75/25, was provided by the faculty of the Department of Macromolecular Science, Fudan University (Shanghai, China). The PLGA had a Mn of 1.81×10^4, a Mw of 3.25×10^4, and a polydispersity (PD) of 1.79.

2.2 Iodine-125 labeling

PLGA was labeled with ^{125}I in chloroform media by a circular heating technique previously established by our laboratory [15]. The Mw and Mn distribution of ^{125}I-PLGA was determined by gel permeation chromatography.

2.3 In vitro degradation measurements

Round, 10 mg ^{125}I-PLGA or PLGA films of 15 mm diameter were suspended in 10 ml of 0.1 M phosphate buffer, pH 7.4 and incubated at 37°C? for 2, 4, 8, 12, and 16 weeks. At each interval, three samples were removed from the buffer, washed with distilled water, and lyophilized. The radioactivity of the residual material was assayed using a gamma scintillation counter (Ri Huan Instrument Factory of ShangHai, China), and the weight was normalized with respect to the zero-time measurement. Experiments were performed in triplicate.

2.4 Subcutaneous implantation of ^{125}I-PLGA

Prior to implantation, 18 Sprague Dawley rats were weighed and identified. The animals were anesthetized by intramuscular injection of ketamine hydrochloride/xylazine. After removing the hair on the back with electric clippers, the surgical site was scrubbed with germicide soap and wiped with 70% alcohol. Veterinary opthalmic ointment was applied to protect the corneas from excessive drying. A skin incision was then made and was large enough to accommodate the test samples. Pockets were formed by blunt dissection. A round ^{125}I-PLGA film (10 mg) was gently placed into each pocket, and the skin was closed using wound clips. All animal procedures were approved by the Animal Care Committee of Jiaotong University (Shanghai, China).

2.5 In vivo degradation measurements

At 2, 4, 6, 8, 10, and 12 weeks after implantation, three rats were randomly selected and anesthetized, samples of cardiac blood (1 ml) were obtained by cardiac puncture before being exsanguinated, then a 4×3 cm^2 section of skin from the back containing residual PLGA material were collected. The mucous surface of the skin tissue was placed directly onto an X-ray film and stored for 24 h at 4°C. Following development of the films, the residual material was removed from the skin, and the radioactivity of this material was assayed using a gamma scintillation counter.

2.6 Determination of body compartment distribution

In addition to the samples collected for the in vivo degradation measurements, the following tissue was collected: heart, liver, spleen, lungs, kidneys, thyroid, stomach, small intestine, large intestine, and skin. All organs, as well as the remaining carcass, was immersed in PBS solution to remove residual blood and then dried in a vacuum oven. The heart, liver, spleen, lung, kidney, and thyroid were homogenized in 5 ml ice-cold 0.25 M sucrose. The stomach, small intestine, large intestine, and the urogenital system were solubilized in 5 M NaOH for 1 h at 50°C. The skin was solubilized in 10 M NaOH. The remaining carcass was incubated in 500 ml of 5 M NaOH at 90°C until the muscle and brain dissolved. The bony portions of the carcass were ground up, suspended in 10 M NaOH, and incubated at 90°C for 30 min. The volume of each fraction was noted. Dissociated ^{125}I was removed from each respective sample by dialysis, which was performed for 24 h against saline solution. One milliliters samples of each fraction, or body compartment, were then assayed for radioactivity. The total radioactivity for each body compartment was calculated by multiplying the average cpm for the 1 ml sample by the total volume of the solution or homogenate. To estimate the total radioactivity in blood, grams of body weight were multiplied by 0.072, and the resulting product was multiplied by the average cpm for the 1 ml sample. Activities found in organs and tissue was expressed as a percentage of the total radioactivity recovered.

2.7 Statistical analysis

The radioactivity of the materials was determined from following equation: $C = C_0 \times e^{-(0.69315 \times t/T)}$, where C was the actual radioactivity, C_0 was the measured radioactivity, T was the half-life of ^{125}I (59.6 days), and t was the time interval between C and C_0. Compiled data were presented as mean ± standard deviation. Residual weight and radioactivity of ^{125}I-PLGA were fitted to a linear curve, and the correlation coefficient was determined. Where feasible, the data were analyzed for statistical significant significance by the student's *t*-test. For all tests, significance was set at the 95% confidence level.

3 Results

3.1 Labeling with Iodine-125

Dialysis of the radioiodinated copolymers against saline solution resulted in the release of a low molecular weight fraction containing ^{125}I, which was removed by repeatedly changing the dialysis solution. The efficiency of ^{125}I labeling, as determined by radioactivity measurements taken before and after dialysis, was approximately 9%, and the radioactivity purity of ^{125}I-PLGA was above 98%. The following values were obtained for the remaining ^{125}I-intact copolymers: Mn = 1.77×10^4, Mw = 2.96×10^4, and PD = 2.01.

3.2 In vitro PLGA degradation

The changes in the weight and radioactivity of residual materials over a period of 16 weeks are presented in Table 1. The data were fitted to the equation $M = 0.00008R + 3.9$ ($P = 0.0088 < 0.05$), where M is the weight of materials (mg) and R is the radioactivity (cpm). *T*-test showed there was no difference in weight between ^{125}I-PLGA and PLGA at any of the time points examined ($P = 0.0501 > 0.05$). For both, weight remained constant over the initial 8 weeks and then dropped dramatically. The changes in radioactivity mimicked the changes in weight. Degradation persisted until almost 16 weeks, at which point the structure had vanished.

3.3 In vivo PLGA degradation

The radioactivity and corresponding weight of residual materials, which were determined using the linear equation obtained from the in vitro degradation experiments, are presented in Table 2. The weight of implanted ^{125}I-PLGA remained constant over the initial 6 weeks, but decreased sharply thereafter. Degradation continued until the radioactivity could no longer be detected at 14 weeks.

As observed from autoradiographic images of ^{125}I-PLGA, the sharpness of the marginal portions of the material first decreased. In addition, some portions of the material broke off. Degradation in the central portions then increased, exceeding the rate of degradation in the marginal portions. This resulted in a change from a round structure to a ring (Fig. 1), and eventually a lune (Fig. 2). At 12 weeks only a small amount of material could be seen.

3.4 Distribution of degraded PLGA

The distribution of radioactivity among selected organs and tissues is presented in Table 3. During the degradation period, the amount of recoverable radioactivity increased among all fractions. The majority of the radioactivity (~60% of total) was distributed to the skin around the material. Almost all the other radioactivity could be found among the blood, liver, and kidneys. Radioactivity steadily appeared in the blood and remained elevated up to 12 weeks

Table 1 The in vitro degradation of ^{125}I-PLGA and PLGA

Materials		Dissolution time in phosphate buffer					
		0 weeks	2 weeks	4 weeks	8 weeks	12 weeks	16 weeks
^{125}I-PLGA	Radioactivity (cpm)	148,870 ± 4,103	133,679 ± 4,566	125,989 ± 6,011	100,893 ± 8,759	70,749 ± 7,539	12,747 ± 3,397
	Weight (mg)	14.9 ± 0.2	14.3 ± 0.3	14.0 ± 0.4	12.8 ± 0.3	11.3 ± 0.8	3.7 ± 0.4
	W/W_0^a (%)	100.0 ± 1.2	95.5 ± 1.7	94.0 ± 2.5	85.7 ± 2.0	75.4 ± 5.1	24.6 ± 3.4
PLGA	Radioactivity (cpm)	–	–	–	–	–	–
	Weight (mg)	14.8 ± 0.2	14.5 ± 0.2	14.1 ± 0.2	13.5 ± 0.4	12.1 ± 0.9	4.3 ± 0.4
	W/W_0^a (%)	100.0 ± 1.0	97.5 ± 1.0	95.3 ± 1.1	90.8 ± 2.4	81.8 ± 6.0	29.4 ± 2.6

[a] Normalized weight of residual PLGA; all data are expressed as mean ± SD of three independent experiments

Table 2 In vivo degradation of implanted ^{125}I-PLGA

	Time after implantation					
	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks
Radioactivity (cpm)	130,343 ± 6,482	124,562 ± 4,949	110,671 ± 12,214	88,933 ± 13,081	57,599 ± 7,141	16,181 ± 5,082
Weight (mg)	14.3 ± 0.5	13.9 ± 0.4	12.8 ± 1.0	10.5 ± 1.0	8.2 ± 0.6	5.2 ± 0.4
W/W_0^a (%)	95.5 ± 3.5	92.4 ± 2.4	85.0 ± 6.5	70.2 ± 7.0	54.9 ± 3.8	34.8 ± 2.7

[a] Normalized weight of residual PLGA; all data are expressed as mean ± SD of three independent experiments

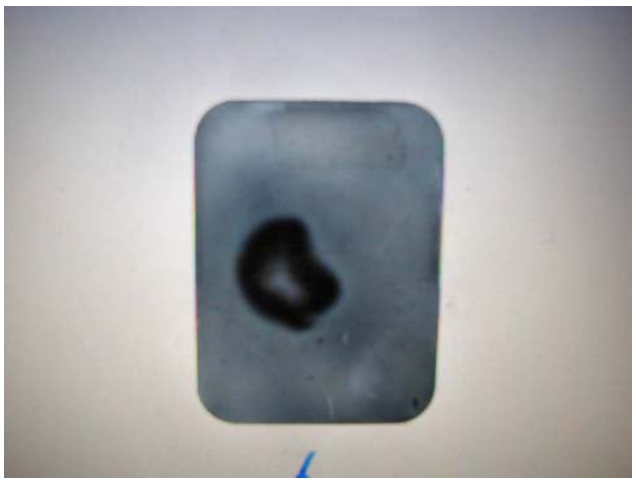

Fig. 1 Autoradiographic images of the PLGA film at 6 weeks after implantation

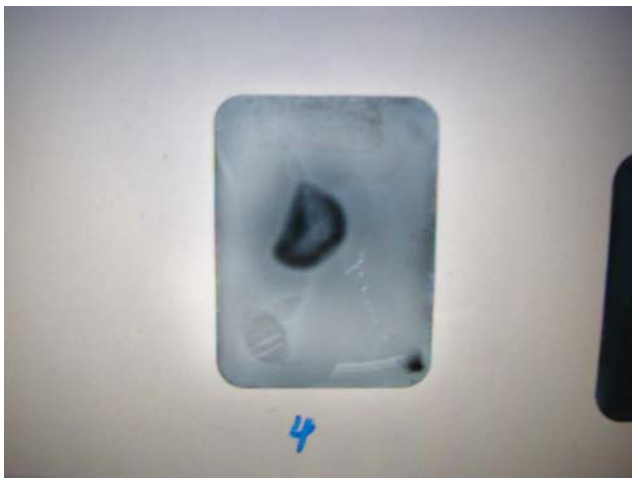

Fig. 2 Autoradiographic images of the PLGA film at 10 weeks after implantation

after implantation. The distribution in kidney began to increase after 6 weeks.

4 Discussion

4.1 Copolymer radiolabeling

In order to reduce the degradation of PLGA during the label process, the reaction temperature and period was limited compared to chloramines T method applied in ^{125}I-labeling of polymers in existence, so the efficiency of ^{125}I labeling (9%) was low; however, after the purification process the radioactivity purity of ^{125}I-PLGA (98%) was high enough to insure the application of this labeling technique in the research of PLGA degradation.

Labeling with ^{125}I is frequently used to study the distribution and metabolism of degradation products. Advantages of this method include its low cost, the high degree of penetrability of gamma radiation released by ^{125}I, as well as the ability to directly measure radioactivity with a gamma counter, without having to separate materials from tissues. In addition, since the thyroid gland only absorbs the dissociative iodine, the radioactivity of thyroid gland could indicate the degree of combination between materials and ^{125}I.

During the labeled process, the PLGA has once been dissolved in the chloroform, so the material with

Table 3 Body compartment distribution of ^{125}I-PLGA

Body compartment	Percent of total recoverable radioactivity[a]					
	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks
Heart	0.2 ± 0.2	0.1 ± 0.0	0.6 ± 0.3	0.7 ± 0.5	0.2 ± 0.0	0.2 ± 0.1
Liver	8.9 ± 1.7	8.3 ± 1.1	10.0 ± 0.9	11.3 ± 1.7	9.8 ± 1.0	7.2 ± 0.6
Spleen	0.5 ± 0.8	0.7 ± 0.5	0.6 ± 0.4	0.9 ± 0.1	0.6 ± 0.4	0.8 ± 0.2
Lungs	0.0 ± 0.0	0.6 ± 0.3	0.3 ± 0.2	0.7 ± 0.5	0.3 ± 0.2	0.5 ± 0.2
Kidneys	4.0 ± 1.1	4.3 ± 1.2	4.8 ± 0.8	4.9 ± 1.2	5.2 ± 0.7	6.2 ± 0.7
Thyroid	0.6 ± 0.4	1.5 ± 0.4	1.1 ± 0.4	1.1 ± 0.4	1.0 ± 0.3	1.4 ± 0.4
Stomach	0.1 ± 0.1	0.3 ± 0.2	0.3 ± 0.2	0.6 ± 0.2	0.3 ± 0.2	0.5 ± 0.1
Small intestine	0.9 ± 0.6	0.7 ± 0.3	0.9 ± 0.3	1.2 ± 0.6	0.9 ± 0.1	1.1 ± 0.2
Large intestine	0.8 ± 0.5	0.5 ± 0.3	0.5 ± 0.3	0.6 ± 0.2	0.9 ± 0.3	1.0 ± 0.4
Skin	67.9 ± 10.1	66.1 ± 6.3	57.4 ± 14.0	54.1 ± 8.4	59.8 ± 5.4	59.5 ± 5.7
Bone	0.0 ± 0.0	0.7 ± 0.4	0.6 ± 0.3	1.1 ± 0.7	0.4 ± 0.1	0.5 ± 0.2
Blood	14.8 ± 2.8	14.1 ± 2.7	22.3 ± 2.4	21.7 ± 5.4	19.6 ± 5.8	19.7 ± 1.9
Residual soft tissue (muscle and brain)	1.1 ± 0.5	1.9 ± 0.7	0.5 ± 0.2	1.1 ± 0.8	1.0 ± 0.3	1.2 ± 0.4

[a] Data are expressed as mean ± SD of three independent experiments

radioactivity is hard for us to be made into any sharp except film. The PLGA film can sometimes used in periodontal guided tissue regeneration for periodontical alveolar bone defect [16], and the subcutaneous implantation process of the PLGA film in this study is similar to the periodontal surgery in many ways.

4.2 In vitro and in vivo PGLA degradation

The finding that PLGA85/15 underwent a drastic decrease in weight after an approximate 8-week period of relatively constant weight is typical of the degradative properties of PLGA [1]. Our results reveal that ^{125}I labeling had little influence on the degradation of PLGA. Due to the capsule of fibrous tissue surrounding the implanted materials and the occasional growth of this tissue into the biodegradable material [17], it is difficult to completely separate the PLGA from the tissue and obtain a reliable weight. Accordingly, published reports of in vivo PLGA degradation are rare. Here, we have not only directly assayed ^{125}I-PLGA using a gamma scintillation counter, but we have also obtained autoradiographic images of the ^{125}I-PLGA material, circumventing the need to separate the material from the tissue.

Calculation of in vivo copolymer weight loss, using the linear equation obtained from in vitro degradation experiments, revealed that decomposition of PLGA occurred more rapidly in vivo than in vitro. This is likely due to the autocatalytic effect of the accumulation of acidic degraded products [18] as well as enzymatic degradation behaviors [19]. And there was actually a vascular reaction to the implants and surgical incisions; the substantial vascularity would also increase the rate of degradation.

Images of ^{125}I-PLGA clearly indicated that the degradation of the central portion of the materials proceeded much more quickly than degradation of the marginal portions. This most probably attributable to the self-catalytic effect of PLGA degradation products, since it would be more difficult to eliminate the degradation products in the central parts of PLGA compared to the marginal parts.

4.3 PLGA biodistribution

In this study, we attempted to characterize principle biodistribution of particles and oligomer fragments released from PLGA in vivo degradation process by ^{125}I radiolabeling. We studied the body distribution of degraded products at six time points up to 12 weeks after the implantation of the ^{125}I-PLGA, and the radioactivity of these degraded products in selected organs and tissues were assayed after removing the dissociative iodine by dialysis.

After subcutaneous implantation, the clearance of degraded particles and fragments from the implantation site was slow, as seen by the high radioactivity in the skin. With the exception of the skin, most of the radioactivity was found in the liver, kidneys, and blood. The concentration of degraded products in the liver and kidneys cannot be attributed to the blood content of these organs, since residual blood was removed by immersing tissue in PBS. So the degraded particles and fragments were selectively concentrated in these organs and tissues, following the release of degradation products from the implantation site into the bloodstream. The existence of these distribution pathways is supported by a previous report describing the tissue distribution of polylactide polymers or copolymer micelles after subcutaneous injection [14].

5 Conclusion

Cumulatively, these results indicate that the clearance of degraded particles and fragments from the implantation site, which is likely subject to self-catalytic effects, is extremely delayed. Moreover, the degraded particles and fragments were selectively concentrated in the liver and kidneys, following release of degraded products into the bloodstream from the implantation site.

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