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Assessment of Biodegradability of Polymeric Microspheres *in vivo*: Poly(DL-lactic acid), poly(L-lactic acid) and poly(DL-lactide-co-glycolide) microspheres

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To confirm a new evaluation technique for biodegradability of biopolymer microspheres in vivo condition, magnetic microsphere system was adopted for tracing the microspheres injected and lodged in mice. Microspheres of poly(DL-lactic acid), poly(L-lactic acid) and poly(DL-lactide-coglycolide)(PLGA) were prepared by solvent-extraction method and their organ distribution and biodegradation in mice was examined. Magnetic microspheres lodged in mice organs were recollected from the homogenates of mice organs with a constant flow magnetic separation apparatus. Recollected microspheres were observed by scanning electron microscopy and also were assayed for their magnetite content by atomic absorption spectrophotometry to evaluate the biodegradability of polymeric microspheres. This method seems to be practical and simple to estimate the biodegradability of biopolymers over the conventional methods.

Key words: Magnetic microsphere, Biodegradability, Poly(DL-lactic acid), Poly(L-lactic acid), Poly(DL-lactide-co-glycolide)

INTRODUCTION

Development of biodegradable polymeric materials for controlled drug delivery system (Dunn *et al.*, 1988; Ogawa *et al.*, 1988; Sato *et al.*, 1988; Thies and Bissery, 1984) has been one of the major advances in recent biomaterial field. Also, biodegradable polymers as carriers for drug delivery system (Juni *et al.*, 1985; Lee, 1988) have attracted much attention for cancer therapy and diagnosis.

The estimation of the exact biodedegradability of biodegradable polymers and their microspheres in the body has been a current topic of researches. The possible methods were radiolabelled method (Yoshioka *et al.*, 1981), fluorescence microscopic method (Lee and Koh, 1987), scanning electron microscopic method (Sanders *et al.*, 1986) and molecular weight determination method such as gel permeation chromatography (Spenlehauer *et al.*, 1989). In this study, magnetite was adopted as a tracer of the microspheres in the body of mice. Magnetite (Fe_3O_4, 10-20 nm in diameter) is non-toxic to normal body constituents and is easily magnetized at a low magnetic field (Widder *et al.*, 1983; Lee *et al.*, 1988a).

Biocompatible and biodegradable polyesters prepared by ring-opening polymerization of glycolide and lactide and their copolymers, e.g., poly(DL-lactic acid) (PDLA), poly(L-lactic acid) (PLLA) and poly(DL-lactide-co-glycolide) (PLGA) were selected as model polymers because they have received attention as noble drug carriers of various drugs (Iwata and McGinity, 1993; Wakiyama *et al.*, 1982), peptide/proteins (Lee *et al.*, 1991; Park *et al.*, 1992) and vaccine (Alonso *et al.*, 1993). Additionally, they are nontoxic, non-tissue reactive and bioerodible. In this study, PDLA, PLLA and PLGA microspheres have been prepared and their biode gradability was determined by analysis of magnetite content in the micro spheres. Their morphological changes *in vitro* and *in vivo* were also examined by scanning electron microscope (SEM).

MATERIALS AND METHODS

Materials

PDLA and PLLA (Polysciences, Warrington, USA),

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PLGA (50 : 50, Boehringer Ingelheim, Ingelheim, Germany) and magnetic fluids (Ferricolloid, HG50, Taiho, Japan) were used as received. All other reagents were of reagent grade.

Preparation of Microspheres

Microspheres containing magnetite were prepared by solvent extraction method (Arshady, 1991; Paventto *et al.*, 1992). Magnetite solution (5 μl) was added to 10% solution of polymer (50 mg) in methylene chloride. And the polymer solution with magnetite was poured into 15 ml of glycerin containing 1% Arlacel A (Sigma, USA). The mixture was emulsified by a mechanical stirrer (TS2010, Curtin Matheson, USA) at 1500 rpm for 10 minutes. The mixture was rapidly added to a 10% isopropyl alcohol and stirred for another 15 min to extract methylene chloride from the dispersed phase. The resulting suspension was centrifuged at 4000 rpm for 10 minutes. The microspheres were washed with 10% isopropylalcohol and dried in vacuum.

Particle Size and Morphological Studies

Particle size of microspheres was measured by the particle size analyzer (Mastersizer/E, Malvern, England) after suspending in distilled water. Microphotographs of microspheres were got from SEM (JCM-35C, Jeol, Japan).

In vitro Studies

After the dried microspheres were dispersed in 15 ml of phosphate-buffered saline solution (PBS, pH 7.4), the suspension was maintained in the shaking incubator at 37.5℃ and shook constantly at the speed of 120 rpm. Fixed amount taken at scheduled intervals was filtered and observed using SEM.

Animal Experiments

Male ICR mice of 20-25 grams were used. All experimental mice were fed with animal chows and tap water *ad libitum*. Three milligrams of microspheres dispersed in 0.3 ml of PBS containing 0.2% Tween 80 (Sigma, USA) was rapidly injected via a tail vein of each mouse. To avoid cluster formation, sonication was performed prior to in jection. Three mice were sacrificed at given intervals and the lungs and liver were isolated. To facilitate homogenation PBS was added to the tissue homogenate.

Recollection of microspheres from mouse organs

Microspheres lodged in the mice lungs and liver were recollected by a constant-flow magnet separation apparatus (Lee *et al.*, 1988b) from the homogenates of the respective organ. Applied magnetic

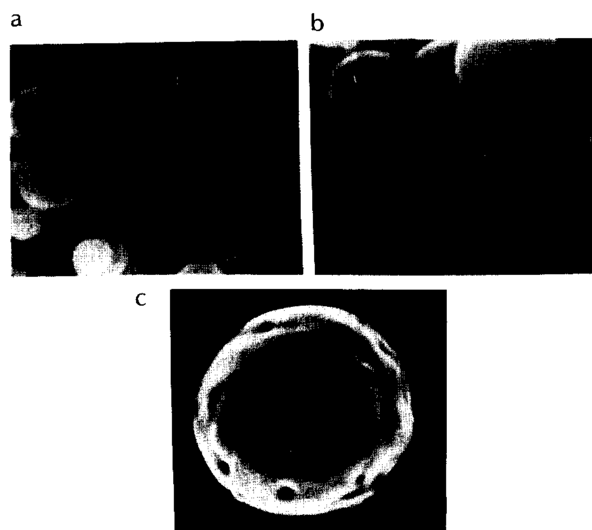

Fig. 1. Scanning electron micrographs of magnetic microspheres prepared by solvent extraction method.
a. PDLA microspheres (×4,000), b. PLLA microspheres (×4,000), c. PLGA microspheres (×4,000)

Table I. Molecular weight of three polymers and the mean particle size of microspheres

Polymers	Molecular weight	Mean diameter, μm± S.D.
Poly(DL-lactic acid) (PDLA)	60,000	3.93± 0.85
Poly(L-lactic acid) (PLLA)	50,000	4.23± 1.02
Poly(DL-lactide-co-glycolide) (PLGA)	40,000	5.52± 0.95

strength and flow rate were fixed on 10 K gauss and 1.5 ml/min, respectively. The microspheres recollected were washed with PBS and dehydrated with absolute ethanol for scanning electron microscopy.

Atomic Absorption Spectroscopic Analysis of Recollected Microspheres

Magnetic microspheres recollected through a constant-flow magnet separation apparatus were analyzed. Microspheres in the chamber were separated by centrifugation at 1500 rpm for 5 minutes. Recovered magnetite was dissolved with hydrochloric acid and assayed by atomic absorption spectrophotometry (PU9200X, Philips, UK) at 248.3 nm.

RESULTS AND DISCUSSION

Molecular weight of polymers used and particle size of microspheres are summarized in Table I. Mean diameter was similar regardless polymers, but size of PLGA microsphere was slightly larger than other microsphere.

The shape of microspheres prepared by solvent extraction precipitation method was spherical as shown in Fig. 1. The unique feature of the micro spheres prepared includes a porous structure. Especially many small pores were noticed on the surface of the PLGA microsphere due to fast removal of solvent(Arshady, 1991; Pavanetto *et al.*, 1992).

The biodistribution of the microspheres in the lungs and liver in 1 day after injection was shown in Fig. 2. By Papisov *et al.*, (1987) magnetic particles are rapidly captured from blood by the liver (80%) and coarse particles more than 5-7 μm diameter also accumulate in the lungs. Particles ranging from 3 to 6 μm were mainly accumulated in the lungs and liver, therefore we measured the % of microsphere only in the lungs and liver. PDLA and PLLA have similar patterns but PLGA microsphere was much accumulated in the lungs because of larger particle size.

Poly(esters) generally degrade in four major stages: a) polymer hydration causing disruption of the primary and secondary struc ture, b) strength loss caused by the rupture of covalent bonds, c) loss of mass integrity resulting in initiation of absorption, and d) solubilization of mass result ing in polymer dissolution and phagocytosis (Linhardt, 1988). Morphological change of micro spheres demonstrated a range of hydrolysis rates. Polylactides hydrolyzed slowly over 4 months at 37℃. Polylactide microspheres were labile to hydrolysis, and hydration followed by polymeric erosion occurred *in vitro* during the dissolution process of substance in the microsphere (Iwata and McGinity, 1993). A neutral hydrophobic substance will tend to work against water uptake and will thus decrease the overall degradation rate (Vert *et al.*, 1991), in contrast, a neutral hydrophilic substance will act in favor of faster degradation. Magnetite was very scarcely released because of the hydrophobic character istics, therefore the microspheres degraded very slowly. Figure 3 shows the scanning electron micrographs of microspheres removed from the dissolution media. After 4 weeks the microspheres remained intact, while after 17 weeks erosion could be discerned. The degradation rate of PLGA microspheres was faster than other microspheres. Poly-

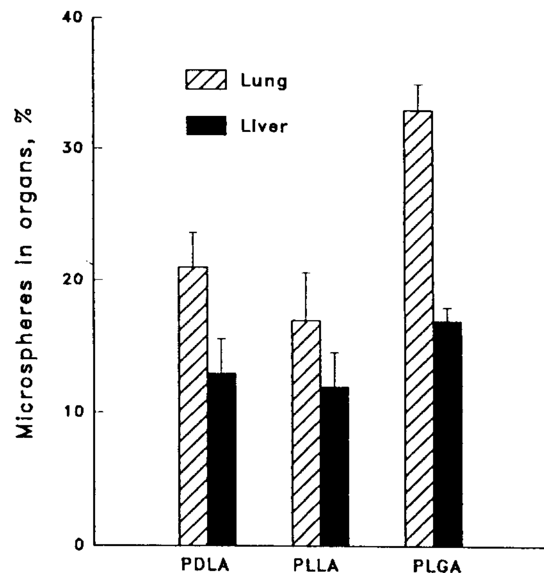

Fig. 2. Initial biodistribution of microspheres in mice organs after 1 day injection.

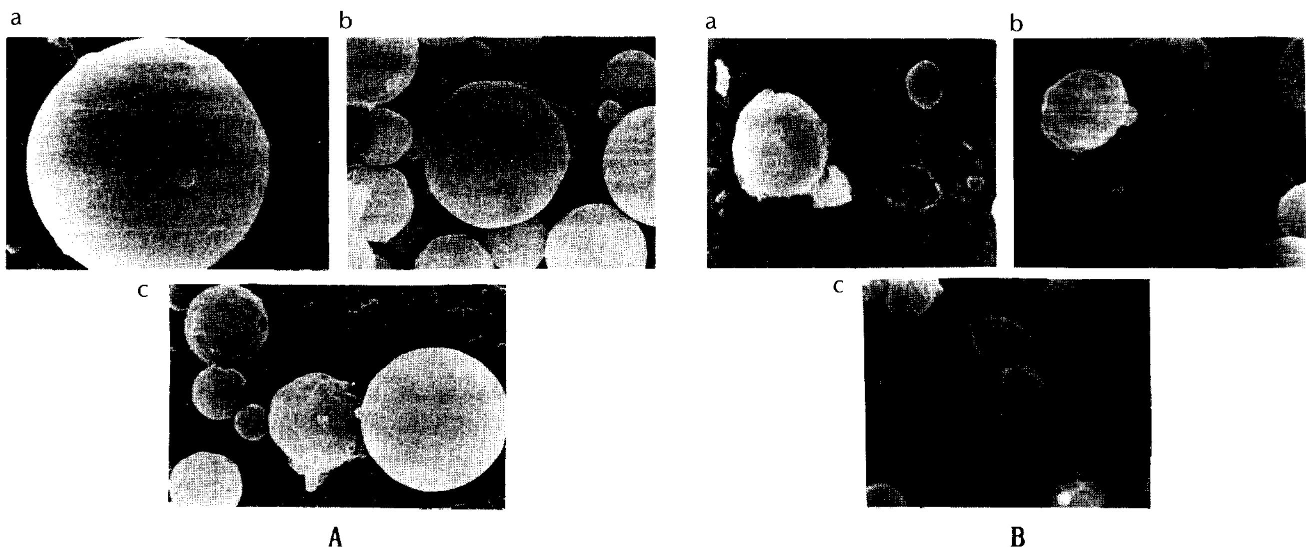

Fig. 3. Scanning electron micrographs of microspheres in vitro.
A: After 4 weeks; a. PDLA (×10,000), b. PLLA (×5,000), c. PLGA (×5,000), B: After 17 weeks; a, PDLA (×4,000), b. PLLA (×4,000), c. PLGA (×4,000)

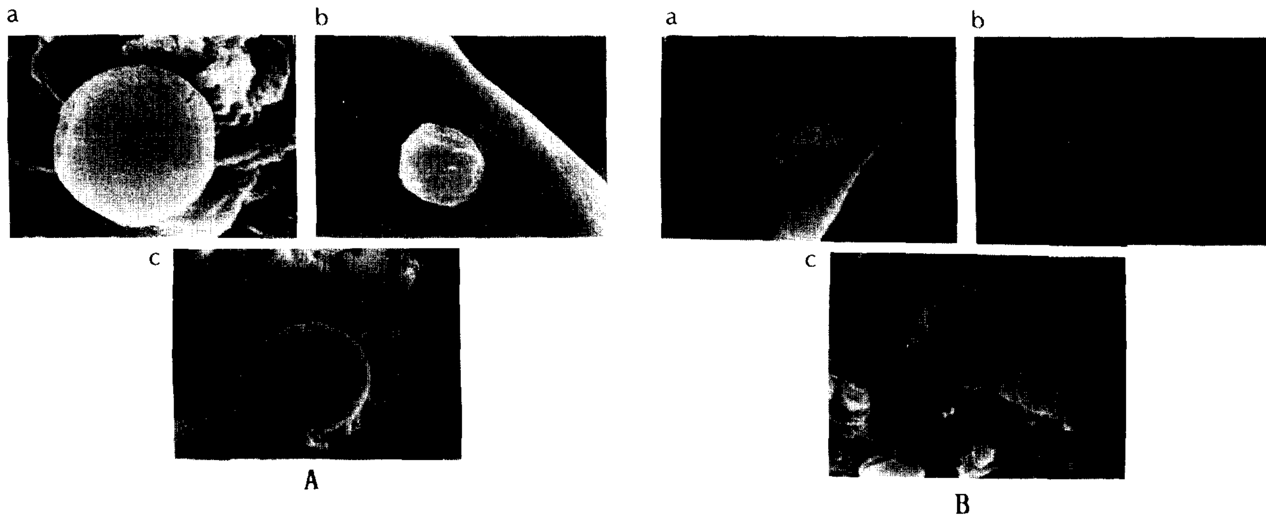

Fig. 4. Scanning electron micrographs of microspheres lodged in mice lung. A: After 1 day; a. PDLA (×10,000), b. PLLA (×3,000), c. PLGA (×4,000), B: After 3 months; a. PDLA (×3,000), b. PLLA (×6,000), c. PLGA (×4,000)

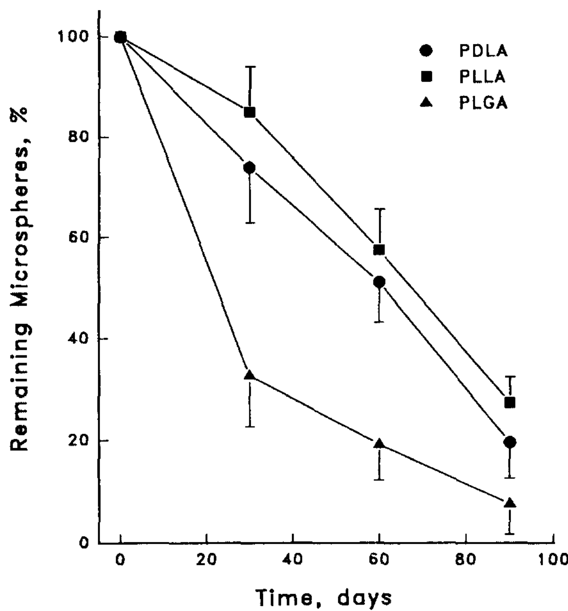

Fig. 5. Biodegradation of microspheres in mice lung.

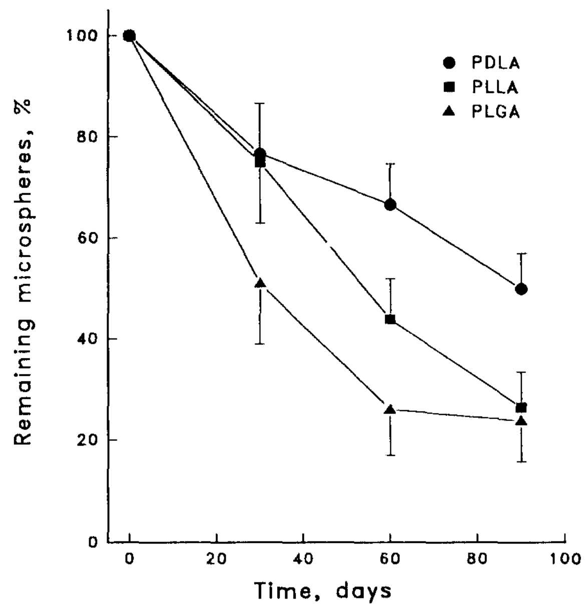

Fig. 6. Biodegradation of microspheres in mice liver.

esters such as PLLA and PLGA hydrolyzed slowly (Wang *et al.*, 1990), but glycolic acid was formed initially at a higher rate than lactic acid, that is, the glycolide units are preferentially hydrolyzed.

The surface of microspheres is degraded more slowly than the core (Spenlehauer, 1989), because of the catalytic effect of hydrosoluble acid monomers and oligomers formed in the polymer matrix during degradation. At the surface of the microspheres, those products are easily eluted by the external fluid while they remain entrapped inside the internal matrix and catalyze the degradation. Figure 4 shows microphotographs by SEM illustrating changes in the surface characteristics of microspheres *in vivo* with time (1 day and 3 months). After 1 day the microspheres remained intact, while after 3 months the matrix had collapsed into fragments. PLGA microspheres degraded at high de gree rate than other microspheres.

The degree of *in vivo* degradation of microspheres was evaluated from the remaining magnetite in microspheres. The amount of magnetite in microspheres recollected in mice lungs and liver was shown in Fig. 5 and Fig. 6, respectively. Magnetite in PLGA microspheres was decreased faster than other microspheres.

The biodegradation of copolymers is a function of the composition of lactic and glycolic acid (Dunn *et al.*, 1988), the 1 : 1 copolymer showing the maximum biodegradation rate *in vivo* (Linhardt, 1988).

In conclusion, the use of magnetite as a tracer brings about many simplifications in handling as well as in the recovery and detection of microspheres in living tissue over the conventional methods such as the radioisotope method and fluoromicroscopic technique. Magnetite can be easily intro ducedinto microspheres physically without altering the chemical and physical properties of the polymer. The recovery of microspheres per animal organ after injection can be simply achieved with a magnet and the assay can be done with a conventional atomic absorption spectrophotometer. We can also prospect the degree of polymer degradation by morphological changes of their microspheres.

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