

INVESTIGATION OF POLYDL-LACTIDE-*b*-POLY(ETHYLENE GLYCOL)-*b*-POLYDL-LACTIDE MICROSPHERES CONTAINING PLASMID DNA *

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Abstract PolyDL-lactide (PDLLA) and the block copolymer, polyDL-lactide-*b*-poly(ethylene glycol)-*b*-polyDL-lactide (PELA) were used as the microsphere matrix to encapsulate plasmid DNA. The PDLLA, PELA, pBR322-loaded PDLLA and pBR322-loaded PELA microspheres were prepared by solvent extraction method based on the formation of multiple $w_1/o/w_2$ emulsion. The microspheres were characterized by surface morphology, mean particle size, particle size distribution and loading efficiency. The integrity of DNA molecules after being extracted from microspheres was determined by agarose gel electrophoresis. The result suggested that plasmid DNA molecules could retain their integrity after being encapsulated by PELA. The PELA microspheres could prevent plasmid DNA from being digested by DNase. The *in vitro* degradation and release profiles of plasmid DNA-loaded microspheres were measured in pH = 7.4 buffer solution at 37°C. The *in vitro* degradation profiles of the microspheres were evaluated by the deterioration in microspheres surface morphology, the molecular weight reduction of polymer, the mass loss of microspheres, the changes of pH values of degradation medium, and the changes of particle size. The *in vitro* release profiles of the microspheres were assessed by measurement of the amount of DNA presented in the release medium at determined intervals. The release profiles were correlation with the degradation profiles. The release of plasmid DNA from PELA microspheres showed a similar biphasic trend, that is, an initial burst release was followed by a slow, but sustained release.

Keywords PolyDL-lactide-*b*-poly(ethylene glycol)-*b*-polyDL-lactide (PELA), Microspheres, pBR322 DNA, Degradation, Release

INTRODUCTION

Gene therapy is a potential method to treat many diseases, especially acquired and inherited genetic diseases. The success of gene therapy is largely dependent on the development of gene delivery systems. Viruses are the most widely used vectors for gene delivery, particularly when high viral loads and repeated administration are needed. Problems such as restricted targeting of specific cell types, limited DNA carrying capacity, recombination, and difficulties of large-scale production of viruses^[1, 2], have led to the development of nonviral delivery systems, such as direct injection of “naked” plasmid DNA *in vivo*, calcium phosphate precipitation, electroporation^[3], particle bombardment^[4], direct intramuscular injection of plasmid DNA^[5], cationic liposome^[6], and synthetic gene carries. Cationic liposomes are the most widely investigated nonviral approach because of their nonspecific ionic interaction and low toxicity^[7–9]. However, cationic liposomes have some limitations because they are unstable when used *in vivo*. Synthesized polymers have many advantages. First, they can be easily synthesized on a large scale; next, their molecules can be designed for our need; third, as to biodegradable polymers, they can be

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degraded *in vivo* and eliminated with the metabolism production; furthermore, with their degradation *in vivo*, the genes in or on the polymers are continuously released in controlled manner. Therefore, many polymeric systems have been studied for *in vivo* application as well as *in vitro* application^[10–12].

As we know, polyDL-lactide (PDLLA) and its copolymers do not cause significant adverse effects in blood and their tissues as the degradation products are eliminated or metabolized easily. However, because of their strong lipophilic property, the affinity between PDLLA/PLGA [poly(DL-lactide-co-glycolic acid)] and water-soluble molecules is not so strong. PEG has been widely used to improve the hydrophilic property of materials and is introduced as the second component connected with PDLLA to form block copolymer PELA. The hydrophilic domains of PELA copolymers could promote the stability of water-soluble molecules, increase the drug and water-soluble molecules loading efficiency and decrease the amount of emulsifier used in the preparation process^[12, 13].

In our previous studies, PELA has been successfully used as protein delivery system^[14, 15]. From those investigations, the simplified preparative procedures of protein-loaded PELA microspheres, the controlled degradation rate of PELA, and higher encapsulated efficiency of protein were obtained. According to the features of PELA, we attempted to use PELA as gene delivery system. We have chosen λ DNA, a linear, double-stranded DNA with higher molecular weight (48.5 kilo bases), as the model genes^[16]. The results showed that λ DNA was able to retain its structural integrity largely and could be prevented from being degraded by DNase after being encapsulated by PELA. That is to say, PELA was able to encapsulate λ DNA molecules. However, the structure of most of plasmid DNA was somewhat different from that of λ DNA. Plasmids were small, extra chromosomal circular molecules, from 2 to around 200 kb in size, which existed in multiple copies within the host *E. coli* cell. They contained an origin of replication, which enabled them to be replicated independently, although this normally relied on polymerase and other components of the host cell's machinery. Plasmid DNA played the important role in gene therapy.

Therefore, in this study, pBR322 DNA was selected as a model gene molecule, which was a subcloning vector containing an anti-tetracycline (T_c) gene and an anti-ampicillin (A_p) gene with molecular weight of 4363 base pairs (bp). We are about to investigate whether the PELA copolymer can efficiently encapsulate the plasmid DNA to prevent it from being degraded by DNase, what are the degradation profiles of plasmid DNA-loaded microspheres, and the release behavior of plasmid DNA from PELA microspheres.

EXPERIMENTAL

Materials

pBR322 DNA (4363 bp) was purchased from Sino American Biotechnology Company (China); polyethylene glycol (PEG, average molecular weight: 6000 Dalton) was from Guangzhou Chemical Reagents Department, China; DL-lactic acid was purchased from Aldrich; DNase I (RNase Free) was purchased from Takara Biotech. Co. (Dalian, China); all other chemicals and solvents were reagent grade or analytical grade.

Synthesis of PELA

Copolymer PELA was synthesized by bulk ring-opening polymerization of lactate/PEG initiated by stannous octoate^[12]. The dried lactide, PEG6000 (6%, *W/W*) and stannous octoate solution in ethyl acetate were placed in a dried and clean glass reactor. The reactor was then purged with nitrogen. The mixture was reacted in an oven at 180°C for 3 h. The crude product was then dissolved in methylene chloride and precipitated from absolute ethanol. The filtered product was washed with absolute ethanol at room temperature and recrystallized from methylene chloride/absolute ethanol three times to give the pure sproduct. The pure copolymer was dried in vacuum at 50°C. The weight-average molecular weight (M_w), determined by gel permeation chromatography (GPC, Waters ALC/GPC244), was 102.2 kDa.

Preparation of Microspheres

The preparation of DNA-loaded PDLLA and PELA microspheres was according to the emulsion-evaporation technique, which was based on the formation of a modified double emulsion $w_1/o/w_2$ reported previously^[14, 15].

Briefly, an aqueous pBR322 DNA solution (100 μ L) was dispersed into a methylene chloride solution (15%, *W/W*) of PDLLA or PELA. The mixture was stirred for 2 min at room temperature to form the primary *w*/*o* emulsion. This emulsion was then syringed into an external water phase (5 mL) and was stirred by a high-speed homogenizer. After adding a certain amount of 6% isopropanol, the emulsion was stirred at a moderate speed at room temperature for 5–6 h. The microspheres were collected by centrifugation (Tomy Seiko Co. Ltd) at 8000 r/min for 10 min, and washed several times with double distilled water. The microspheres were then lyophilized overnight and stored at -20°C .

Characterization of the Microspheres

The microspheres were sized by laser diffractometry using a Malvern 2000 laser sizer (Malvern Instruments PC6300, Malvern, England). The average particle size was expressed as the volume mean diameter in μm . The surface morphology of the microspheres was evaluated by scanning electron microscopy (SEM, Amray). A monolayer of dry microspheres was mounted on an aluminum stub using double-sided carbon tape. The sample was coated with a 10 nm thick palladium/gold film using a sputter coater. The coated sample was examined at an electron acceleration voltage of 20–25 kV. The level of residual methylene chloride within microspheres was detected by gas chromatography (GC, Shanghai Analytical Instrument, China), and compared with a set of standard samples with known amount of methylene chloride.

Extracting the DNA from microspheres and assaying the amount of the extracted DNA revealed the DNA loading of the microspheres. In detail, 200 mg of PELA microspheres were dissolved in 1500 μ L of methylene chloride and 500 μ L of 10 mmol/L Tris-EDTA buffer (pH = 7.4) was added to extract DNA. After centrifugation at 3000 r/min for 5 min, the aqueous layer was transferred into a fresh tube. The concentration of DNA was assessed by fluorescence spectrophotometer (Shimadzu, RF-5400, Japan) dying with ethidium bromide. The stability and topology of DNA were evaluated by 0.8% agarose gel electrophoresis.

***In vitro* Degradation Study of the pBR322-Loaded PELA Microspheres**

Microspheres (300 mg) were suspended in 10.0 mL of 0.1 mol/L phosphate buffered saline (PBS, pH = 7.4). The mixtures were kept in a Thermostated Shaker Water Bath (Jiangsu Taicang Medical Apparatus Co., China) at 37°C with 60 r/min. The degradation medium was removed at a predetermined time from the vessel and replaced with same volume of fresh PBS. At predetermined intervals, triplicate samples for each kind of microspheres were recovered, rinsed with distilled water to remove any residual buffer salts and dried to constant weight in a vacuum desiccator at room temperature.

The degree of degradation was estimated from the decrease in molecular weight, from the loss-in-mass, and the morphological changes of microspheres.

The molecular weight of the polymers was determined by gel permeation chromatography (GPC) in tetrahydrofuran (THF) at 30°C with an Ultrastayragel lineal column (Waters, USA) and a refraction index detector (R401, Waters), and calibrated with polystyrene standards. Mass loss was calculated by comparing the net weights of microspheres at predetermined intervals with the initial net weight of the microspheres. SEM was used to observe the surface morphological changes.

***In vitro* Release Study of the pBR322-Loaded PELA Microspheres**

The release profile of the pBR322-loaded PELA microspheres was determined in 0.2 mol/L phosphate buffer saline (PBS, pH = 7.4). 250 mg of microspheres were incubated in 5.0 mL of PBS, shaking at $(37 \pm 0.5)^{\circ}\text{C}$, 60 r/min. At predetermined intervals, 1.0 mL of release medium was removed after centrifugation (5 min, 4000 r/min), and replaced with an equal volume of fresh PBS. The concentration of released pBR322 DNA was determined by fluorescence spectrophotometry (RF-5000, Shimadzu).

DNase I Digestion Study

The study of DNase I digestion was according to the method reported by Sisay *et al.*^[17]. Briefly, 200 mg of microspheres was suspended in 4 mL of 10 mmol/L Tris-HCl buffer (pH = 8.0) containing 3 mL of 10 mmol/L MgSO_4 , and 2 μ L of DNase I (5 $\mu\text{g}/\mu\text{L}$) in 0.69% of NaCl. The mixture was incubated for 30 min at 37°C . After

digestion, samples were washed 3 times with 1000 μL of fresh Tris-EDTA buffer (pH8.0), and assessed by agarose gel electrophoresis (0.8%, W/V).

RESULTS AND DISCUSSION

Characterization of Microspheres

The synthesized PELA copolymer with a molecular weight of 102 kDa was used as the microsphere matrix. The microspheres were prepared by the emulsion-evaporation method. Figure 1 indicated the scanning electron microscopy (SEM) of the PELA microspheres. It showed that the spherical shape and the distribution of pBR322-loaded PELA microspheres were not perfect. The microspheres seemed to be ellipsoid, and irregular particles still existed. But the particle size of pBR322-loaded PELA microspheres was smaller than that of PDLLA.

It is well known that PDLLA was a kind of hydrophobic polyester, while DNA molecules were hydrophilic. The affinity between PDLLA and DNA molecules was so weak that it was more difficult to encapsulate DNA molecules to form smaller size microspheres. The introduction of hydrophilic PEG domains into the backbond of PDLLA improved the hydrophilic property of copolymer, following which the affinity between copolymer and DNA molecules was ameliorated and then the DNA-loaded PELA microspheres were more easily formed. The improved phase compatibilities between copolymer and DNA molecules were also proved by the increased encapsulating efficiency, which is shown in Table 1. The pBR322 loading of 0.5% with loading efficiency of 61% was achieved for PELA microspheres, while pBR322 loading efficiency of 45% was obtained for PDLLA microspheres prepared by the similar process.

Table 1. The characters of pBR322 plasmid DNA-loaded PDLLA and PELA microspheres

	PDLLA	PELA
M_w^a	93.5 kDa	102 kDa
Mean diameter (μm) ^b	3.8	2.4
Diameter uniformity ^b	0.62	0.58
DNA loading (%)	0.5	0.5
Loading efficiency (%)	45	61

^a Measured by GPC; ^b Measured by laser diffractometer

On the other hand, the molecules of PELA appeared somewhat negative under neutral conditions compared with PDLLA because of the ether bonds of the copolymer, while each phosphate group had a single negative charge under neutral conditions in DNA molecules. The electrostatic repulsion between copolymers and DNA molecules detracted from the formation of perfect microspheres. So that the DNA-loaded PELA microspheres were with pits on the surface, the distribution of particle size was not even and the shape of the microspheres seemed to be ellipsoid.

From the analysis of laser diffraction, the mean particle diameter of pBR322-loaded PELA microspheres was 2.4 μm . According to the previous reports, the size of the microspheres made of polymers with larger molecular weight was larger than that of microspheres made of polymers with smaller molecular weight. Otherwise, under the same preparative condition, the mean particle diameter of pBR322-loaded PDLLA microspheres was 3.8 μm , even if the molecular weight of PELA (102 kDa) was larger than that of PDLLA (93.5 kDa). The result was another proof that the improved hydrophilic property of polymer was beneficial for microsphere formation. Moreover, the introduction of PEG into the backbone of PDLLA improved the softness of the molecular chain, which increased the mobility of molecular chain, so that it was easier to fabricate PELA microspheres than PDLLA ones. In gene therapy, the particle size was very important. One of the reasons was that smaller particle size could efficiently prevent capillary clogging. And we expected phagocytes to take up the microspheres. It has been reported that in order to be taken up by phagocytes, the particle size of microspheres must be in the range of 10 μm ^[17]. Another reason was that microspheres with size 1–5 μm could be targeted to certain tissues, such as the intestinal Payer’s patches, liver and spleen after oral administration.

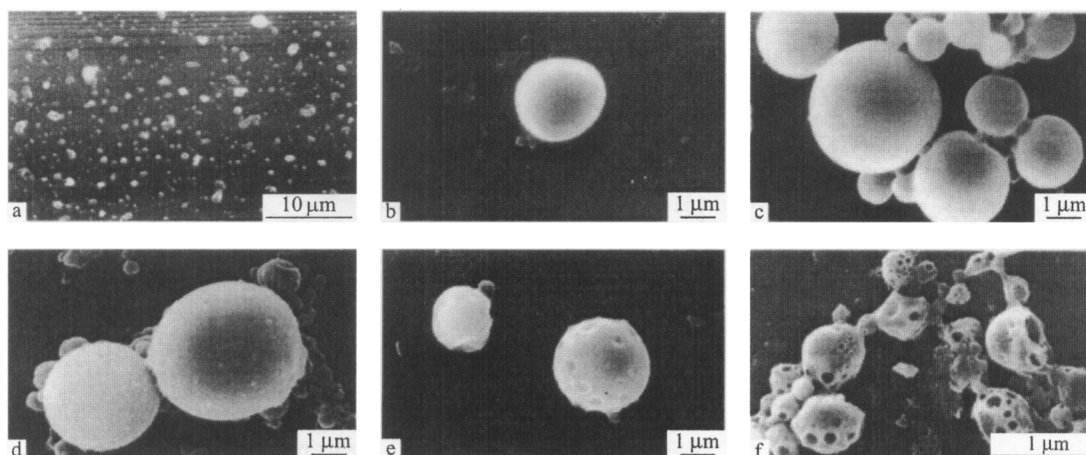


Fig. 1 The surface morphology of microspheres

a) The distribution pattern of pBR322-loaded PELA microspheres; b) pBR322-loaded PELA microspheres; c) Blank PELA microspheres; d) Blank PDLLA microspheres; e) pBR322-loaded PELA microspheres after 20 days' incubation; f) pBR322-loaded PELA microspheres after 80 days' incubation

The homogenization speed was an important factor to the particle size. Generally, the higher the homogenization speed the smaller was the particle size. Nevertheless, the molecules of DNA were sensitive and easily damaged by shearing forces. Thereby the homogenization speed had to be chosen carefully. In our investigations, the initial homogenization speed was less than 2000 r/min for 2 min, and the residual methylene chloride was extracted at speed of 500 r/min. When supersonic shock waves were used to distribute the initial emulsion for 1 min, the microspheres diameter was under 1 μm , but most of the plasmid DNA was damaged according to the assay of agarose gel electrophoresis (shown in Fig. 2). When the homogenizer speed for extracting solvent was too high, the morphology of microspheres was irregular. On the contrary, when the extraction speed was too low, for instance, below 100 r/min, the particle size was larger. Because the higher extraction speed resulted in a higher shearing force, which made the non-solid polymeric matrix move towards the direction of the shearing force, irregular polymeric microspheres were obtained. However, if the extraction speed was too low, the unsolid polymer microspheres would aggregate to form larger new microspheres for the inter-molecular forces and the collision of the microspheres. Of course, there are many other factors influencing the particles size, which we have discussed in Ref. [18].

Figure 2 was the result of agarose gel electrophoresis. It showed that pBR322 DNA extracted from microspheres retained their integrity. The residual methylene chloride presented in microspheres was below $\delta = 20$, which was evaluated by gas chromatography. The obtained value was lower than the limited value according to the USP XXIII requirement (*i.e.* $\delta = 500$ for methylene chloride)^[19].

DNase I Digestion Study

As expected, the PELA could protect pBR322 from being degraded by DNase I. In Fig. 2, lane 3 was the free plasmid DNA after being digested by DNase I; lane 5 was the pBR322 encapsulated in PELA microspheres after exposure to DNase I. This indicated that free DNA treated by DNase was degraded completely, while DNA encapsulated in microspheres was able to remain its integrity after exposure to DNase I.

In vitro Degradation of Plasmid DNA-Loaded Microspheres

The *in vitro* degradation of microspheres was evaluated by the deterioration in microsphere surface morphology, the molecular weight loss of polymer, the mass loss of microspheres, the changes of pH values of degradation medium, and the changes of particle size.

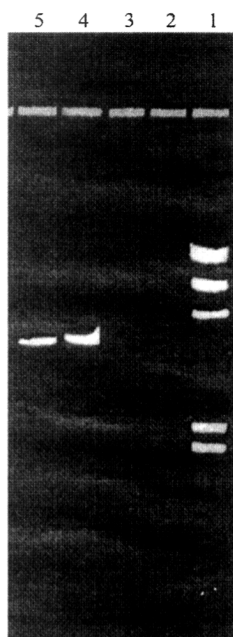


Fig. 2 The agarose gel electrophoresis of DNA

Lane 1, marker; Lane 2, distributed by supersonic for 1 min; Lane 3, free pBR322 after being digested by DNase I; Lane 4, pBR322 extracted from microspheres; Lane 5, the encapsulated pBR322 after being digested by DNase I

With increasing incubation time, the deterioration in microsphere surface morphology was becoming apparent, which is shown in Fig. 1(e) and (f). The deterioration was ascribed to the reduction in the molecular weight of polymeric microspheres. Figure 3 shows the molecular weight loss and the mass loss of microspheres. The molecular weight of all the microspheres decreased with incubation time, but the PDLLA microspheres showed the steadiest decrease. The molecular weight of plasmid DNA-loaded PELA microspheres decreased more rapidly than that of PELA microspheres. Moreover, all the microspheres made of PELA showed the same reduction trend: more rapid initially, slower but more sustained afterwards. In contrast, the reduction of molecular weight of PDLLA microspheres was at a constant rate. After 80 days of degradation, the molecular weight showed about 41.5% loss of blank PELA microspheres, and 50.3% loss of molecular weight was obtained for the plasmid DNA-loaded microspheres, while PDLLA microspheres only lost approximately 28.6% of molecular weight. In Fig. 3(b), no apparent mass loss could be detected for PDLLA and PELA microspheres during the initial days. The plasmid DNA-loaded microspheres, on the other hand, were observed to undergo a slower mass loss at first, faster afterwards. After 80 days, the residual percents of microspheres were 91.7%, 87.5%, and 65.6% for PDLLA, PELA, and pBR322-loaded PELA microspheres, respectively. These results suggested that the degradation of plasmid DNA-loaded PELA microspheres was faster than that of blank PELA microspheres, and far faster than that of PDLLA microspheres.

As we have discussed above, the hydrophobic property hinders the permeation of water. It resulted in delayed hydration, and slower hydrolysis for the PDLLA. And the smaller microspheres degraded more rapidly than larger ones. As a result, the reduction of molecular weight and the mass loss for PELA microspheres appeared more rapid than for PDLLA. From Fig. 3, both the molecular weight and the polymer mass of the plasmid DNA-loaded PELA microspheres showed faster reduction than those of blank ones. It obtained that plasmid DNA-loaded PELA microspheres degraded more rapid than blank PELA microspheres. As a reason it may be considered that the encapsulation of DNA within microspheres improved the hydrophilic property of PELA further.

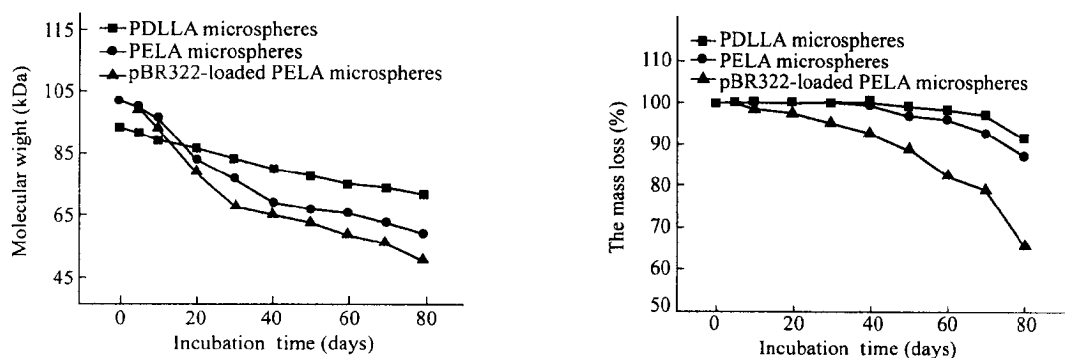


Fig. 3 The reduction of the molecular weight and the mass loss of microspheres with incubation time *in vitro*

(a) The reduction of molecular weight of microspheres with incubation time

(b) The mass loss of microspheres with incubation time

From Fig. 3, it was also found that the mass loss lagged behind the molecular weight reduction because the mass loss did not occur until the fragments of polymers or oligomers, which were soluble in degradation medium, were formed, but the molecular weight loss began when the microspheres were dispersed in PBS. That is to say, the scission of bonds began as soon as the microspheres were exposed to PBS. Furthermore, the PDLLA microspheres showed the most obvious phenomenon of lag, because of the worst hydrophilic property of the PDLLA microspheres. This proved that the PELA copolymer hydrolysis should contribute to the bulk degradation of polymer, just like PDLLA^[20]. It was required that the surface layer hydrolysis and erosion preceded the molecular weight loss. The ameliorated hydrophilic property could also be proved by the difference of diameters measured by SEM and laser diffractometry respectively. The diameters measured by laser diffractometry were 3.0, 2.1, and 2.4 μm for PDLLA, PELA, and plasmid DNA-loaded PELA microspheres, respectively, while the diameters measured by SEM were 2.8, 1.8, and 1.9 μm , and the rates of increase were 6.7%, 14.3%, and 20.8%, respectively. This was because the microspheres were dispersed in water for laser diffractometry, while microspheres were required in the vacuum-dried state for SEM measurement.

The degradation of PDLLA generated acid substances^[20], which acidified the degradation medium generally, whereas the acid conditions were harmful to the released DNA molecules. However, the PELA copolymer was able to support a more stable environment for DNA. By virtue of the hydrophilic nature of PEG in PELA, the swelling of PELA was easier than that of PDLLA. The swelling led to a rapid exchange of ions, which made a neutral pH environment inside the microspheres. The neutral environment prevented aggregation and denaturation of DNA molecules. Figure 4 shows the pH value changes of the degradation medium with incubation time. It was found that the pH increased slightly rather than decreased during the initial 20 days. On the 20th day, the pH was at its highest, 7.59. After 20 days, the pH values decreased gradually. This coincided with the accelerated degradation of polymer. From 20 days, the mass loss was accelerated (shown in Fig. 3b). It indicated that the larger fragments of polymer had little influence on the pH value of the degradation medium, only soluble fragments contributed to the pH of the medium.

The changes of particle size and particle size distribution of microspheres during incubation time were shown in Fig. 5. The particle size of the microspheres increased gradually, but no obvious increase was observed during the initial days, while the particle size distribution was widening with incubation time. The change rates of both of them for plasmid DNA-loaded PELA microspheres were larger than for blank PELA microspheres. At the mean time, the changes of average particle size lagged behind the changes of particle size distribution. Figure 5 coincided with the degradation profiles. It was proved again that time was need for water to permeate into polymeric matrix, and the ameliorated hydrophilic property reduced the time. As we have discussed above^[16], the hydrophilic property of DNA-loaded PELA microspheres was better than that of PELA

microspheres, so the size change of plasmid DNA-loaded PELA microspheres was more violent than that of PELA ones.

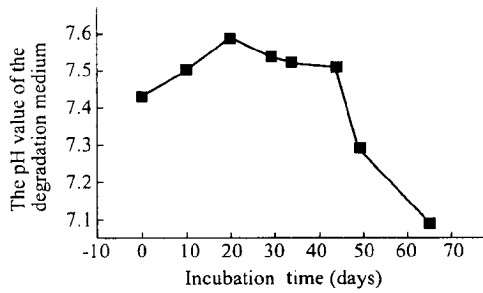


Fig. 4 The changes of the pH value of degradation medium with incubation time *in vitro*

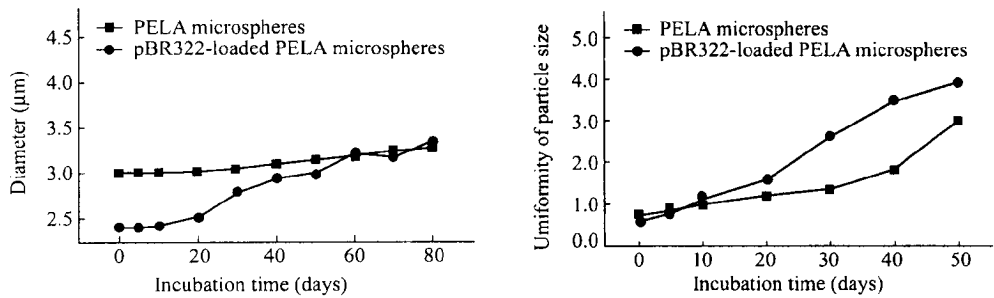


Fig. 5 The changes of particle size and particle size distribution *in vitro* with incubation time
a) The changes of particle size incubation time
b) The changes of particle size distribution incubation time

In vitro Release of Plasmid DNA-Loaded Microspheres

In this investigation, the release of plasmid DNA from PELA microspheres revealed a similar biphasic trend, just like the release of λ DNA from the PELA microspheres^[16], that is, an initial burst release was followed by a slow, but sustained release. The electrostatic repulsion forces between DNA molecules and polymer and among DNA molecules promoted the diffusion of the DNA molecules into the degradation medium. When the DNA molecules within the microspheres passed through the polymer matrix, passages were gradually formed in the microsphere matrix, after which the sustained release commenced. It has been reported that systems based on PDLLA and its copolymer in an aqueous environment underwent hydration followed by bulk erosion^[18]. That is to say, the release of DNA from the microsphere delivery system depended on both erosion and diffusion. A variety of factors could influence DNA release from microspheres, including chemical structures and molecular weight of the polymer, particle size and morphology of microspheres, loading amount and the solubility of DNA, and the concentration of release medium. No simple mathematical model could therefore be applied to predict or quantify release rates in these systems.

In Fig. 6, it is seen that plasmid DNA molecule release commenced before the molecular weight reduction and mass loss were clearly observed. The release of plasmid DNA from PELA microspheres was characterized by a release of 15.6% approximately in the initial 10 days. But the amount of burst release for plasmid DNA from PDLLA microspheres was 60.2% approximately. Actually, the burst release of plasmid DNA from PELA microspheres was less prominent than from PDLLA ones.

After that, the swelling and hydrolysis of polymer ensured. The DNA molecules in deeper regions would not be released until the pores or passages passing through the microspheres were formed and water entered the inner microspheres. Up to 40–50 days, the release of DNA increased which was in close agreement with the breakdown of polymeric mass loss and the increase of particle size. Therefore, it is considered that when the

molecular weight reduction became significant and the mass loss was remarkable, an increase of the formation of passages was achieved, and the higher release rate was observed. Consequently, it was assumed that the release behavior of PELA microspheres might be according to the swelling mechanism in addition to the diffusion and erosion mechanism. PELA has this shown its potential as a novel polymeric matrix for gene delivery system.

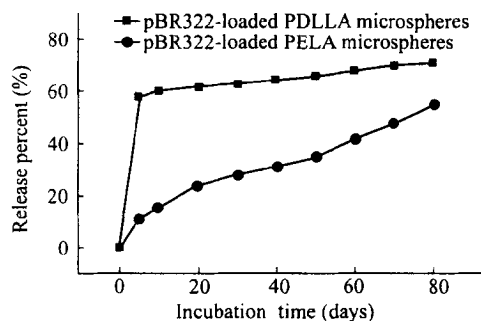


Fig. 6 The *in vitro* release of DNA from microspheres

CONCLUSION

We have used the $w_1/o/w_2$ double emulsion-solvent extraction method to encapsulate pBR322 plasmid DNA with PELA. The mean particle size of pBR322 plasmid DNA-loaded PELA microspheres was about 2.4 μm . After being encapsulated by PELA, pBR322 DNA molecules were able to retain their structural integrity. Furthermore, being encapsulated by PELA can prevent plasmid DNA from being degraded by DNase. The loading efficiency of plasmid DNA-loaded PELA microspheres was 61%. From these results, we can consider that PELA was able to encapsulate plasmid DNA. The *in vitro* degradation profiles of different microspheres were investigated. The degradation of plasmid DNA-loaded microspheres was faster than that of blank PELA microspheres, while the blank PELA microspheres degraded more rapidly than PDLLA ones. It proved that the introduction of hydrophilic PEG domains into PDLLA improved the hydrophilic property of PDLLA, and promoted the degradation of polymer. During degradation, the molecular weight and the mass of PELA were reduced, but the mass loss lagged behind the reduction of molecular weight. The particle size and the particle size distribution increased with incubation time. The pH value of the degradation medium increased initially and then decreased slowly. The *in vitro* release profile of plasmid DNA-loaded PELA microspheres was in agreement with the degradation profile, and showed a biphasic form: an initial burst release was followed by a sustained release.

REFERENCES

- 1 Crystal, R.G., Science, 1995, 270: 404
- 2 Tripathy, S.K., Blavk, H.B., Goldwasser, E. and Leiden, J.M., Nat. Med., 1996, 2: 545
- 3 Wadhwa, M.S., Knoell, D.L., Young, A.P. and Rice, K.G., Bioconjugate Chem., 1995, 6: 283
- 4 Conry, R.M., Widera, G., Lobuglio, A.F., Fuller, J.T., Moore, S.E., Barlow, D.L., Turner, J., Yarg, N.S. and Currer, D.T., Gene Ther., 1996, 3: 67
- 5 Tripathy, S.K., Svensson, E.C., Black, H.B., Goldwasser, E., Margalith, M., Hobart, P.M. and Leiden, J.M., Pro. Natl. Acad. Sci. U. S. A., 1996, 93: 10876
- 6 Gao, X. and Huang, L., Gene Ther., 1995, 2: 710
- 7 Felgner, J.H., Kumar, R. and Sridher, J., Biol. Chem., 1994, 269: 2550
- 8 Farhood, H., Serbina, N. and Huang, L., Biochem. Biophys. Acta., 1995, 1235: 289
- 9 Zabner, J., Fasbender, A.Z., Moniger, T., Pollinger, K.A. and Welsh, M.J., J. Biol. Chem., 1995, 270: 18997
- 10 Mumper, R.J., Wang, J., Claspell, J.M. and Rolland, A.P., Proceed. Intern. Symp. Control. Rel. Bioact. Mater.,

1995, 22: 179

- 11 Haensler, J. and Szoka, F.C., *Bioconjugate Chem.*, 1993, 4: 372
- 12 Deng, X.M., Xiong, C.D. and Cheng, L.M., *J. Poly. Sci., Polym. Lett.*, 1990, 28: 411
- 13 Li, X.H. and Deng, X.M., *J. Appl. Polym. Sci.*, 1997, 66: 503
- 14 Deng, X.M., Li, X.H., Yuan, M.L., Xiong, C.D., Huang, Z.T., Jia, W.X. and Zhang, Y.H., *J. Controlled Release*, 1999, 58: 123
- 15 Zhou, X.B. and Deng, X.M., *React. Funct. Polym.*, 2002, 51: 93
- 16 Deng, X.M., Liu Y., Yuan, M.L. and Li, X.H., *Appl. Poly. Sci.*, 2002, 86 (10): 2557
- 17 Yilmaz, C., Byung, W.H., Sisay, G., Shamim, A. and Patrick, P.D., *Pharm. Res.*, 1999, 16(4): 509
- 18 Liu, Y. and Deng, X.M., *J. Control. Release*, 2002, 83 (1): 147
- 19 Bitz, C. and Doelker, E., *Int. J. Pharm.*, 1996, 131: 171
- 20 Zhu, J.H., Shen, Z.R., Wu, L.T. and Yang, S.L., *J. Appl. Polym. Sci.*, 1991, 43: 2099