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Development of interferon alpha-2b microspheres with constant release

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ABSTRACT

Interferon alpha-2b (IFNα-2b) is an important immune regulator used widely in clinic. However, frequent subcutaneous injection and substantial toxicity decrease patients' compliance. So, drug delivery with more precisely controlled drug release is urgent for IFNα-2b. Microsphere is a promising sustained drug delivery system, which has been studied widely for delivery of proteins. However, it was found difficult to keep proteins' activity and guarantee complete release. In this study, we solidified IFNα-2b as microparticles firstly by co-lyophilizing it with gelatin and $ZnSO_4$. Microspheres were then prepared. The preparing procedure and formulation were optimized with encapsulation efficiency and in vitro release as main parameters. Finally, the microspheres were prepared by S/O/W method with microparticle size about 5 μm and PEGT/PBT-PLGA (9:1, w/w) as matrix material. The diameter of microspheres was 28.94 μm, the encapsulation efficiency was 86.01%, the burst release was 16.69%, the cumulative release was 83.06% at 23th day, and IFNα-2b was released from microspheres with a zero-order profile. These microspheres also demonstrated sustained and steady release for about 13 days in rats. In conclusion, the procedure and formulation used in this study were supposed to be successful to keep IFNα-2b active and released constantly and completely.

1. Introduction

Interferons, which are secretory proteins induced by viral and other stimuli, exhibit many biological activities, including activities against a variety of RNA and DNA viruses, potent immunomodulatory effects and antiproliferative activity against malignant cells (Zoon et al., 1986; Bordens et al., 1997). Now, genetically engineered interferon alpha-2b (IFNα-2b) produced from microorganisms (e.g. *Escherichia coli*) is used widely in clinic, especially in the therapy of leukemia, multiple myeloma, carcinoma, hepatitis B and hepatitis C (Kirkwood et al., 2002; Bayraktar et al., 1996).

In the early phase of the administration of interferons, frequent subcutaneous injection was necessary to keep interferons at effective concentration. However, fluctuant drug level was inevitable. With a relatively small therapeutic index, interferons showed substantial toxicity at levels above the maximal therapeutically effective concentrations. Thus, precisely controlled delivery is necessary for interferons to achieve therapeutic effects and avoid side effects to the greatest degree.

According to the urgent clinical requirements, alternative formulations to increase efficacy and decrease toxicity have been investigated in the last few years including PEGylated interferon, which is now available in market (PEG-Intron®). Through conjugating with monomethoxy polyethylene glycol (PEG), IFNα-2b can reach maximal serum concentration at 15–44 h after initial dose and sustain for up to 48–72 h after single subcutaneous dose. It seems convenient for patients to administrate drugs once a week subcutaneously for one year. However, clinical trial showed that 10–14% of patients receiving PEG-Intron, alone or in combination with REBETOL, discontinued therapy compared with 6% treated with INTRON A, a recombinant interferon alpha-2b for injection in market, alone and 13% treated with INTRON A in combination with REBETOL. The most reasons for discontinuation of therapy were related to known interferon effects of sychiatric, systemic (e.g. fatigue, headache) or gastrointestinal adverse events (http://www.rxlist.com/peg-intron-drug.htm 2009). It seems that PEGylated IFNα-2b is easier to induce side effects than IFNα-2b.

Recently, more and more attempts have been put on sustained drug delivery system composed of biodegradable polymers to control release of interferons. Among them, poly(lactic-glycolic acid) (PLGA) microparticles (Diwan and Park, 2003; Tracy et al., 2000; Manish and Tae, 2003), gelatin microparticles (Tabata and Ikada, 1989; Yoshikawa et al., 1999) and starch microparticles (Degling et al., 1993) have all been investigated, and the release time of interferons can be prolonged up to 28 days in vitro (Sánchez et al., 2003), which is much longer than PEGylated interferons. Although all of those developments guarantee the sustained release of interferons, it seems hard to keep the activity and complete release of these proteins during the whole preparative and release period due

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to the instability of proteins or the physical and chemical interactions during the preparation and release of microspheres (Sinha and Trehan, 2003).

In this paper, solidified IFNα-2b, instead of liquid form, was used in the microencapsulation process to make it more stable and improve its release from microspheres. Gelatin, a kind of protein, was added into the microspheres to protect IFNα-2b from denaturation. $ZnSO_4$ was also added, which is also beneficial to the activity of IFNα-2b (Bernstein et al., 2002). These microspheres showed constant and complete release of IFNα-2b both in vitro and in vivo.

2. Materials and methods

2.1. Materials

Interferon alpha-2b (IFNα-2b, 1.329 mg/ml, 2.1×10^8 IU/ml) was obtained from Shenzhen Neptunus Interlong Bio-Technique Company (Shenzhen, China). Poly(D,L-lactide-co-glycolide) copolymer (PLGA, lactide:glycolide molar ratio is 50/50 and molecular weight is 10,000 Da) was purchased from Birmingham Polymers (Birmingham, USA). Poly(ethylene glycal/butylenes terephthalate) (PEGT/PBT, weight ration of PEGT/PBT is 70/30 and PEG molecular weight is 600 Da) was obtained from Capital Medical University. Polyvinyl alcohol-124 (PVA 124) was produced by Beijing East Ring Union Chemical Plant. Gelatin was provided by Qinghai Gelatin Factory. Lecithin from yolk was purchased from Shanghai Fine Chemical Co., Ltd. Petroleum ether with boiling point between 60 and 90 °C was from Beijing Chemical Fine Chemicals Co., Ltd. Purified cottonseed oil was from Wudi Yatai Import & Export Co., Ltd.

2.2. Preparation of microspheres

2.2.1. Preparation of gelatin microparticles

5 mg gelatin was dissolved in 1 ml pH 6.8 phosphate buffered saline (PBS) at 40 °C and then IFNα-2b was added into above solution at ambient temperature. The final solution was obtained by adding equal volume of 5% (w/v) $ZnSO_4$ and frozen at −50 °C. The frozen solution was then freeze-dried at −20 °C for 2 h, 0 °C for 12 h, 10 °C for 2 h. Gelatin microparticles with IFNα-2b were obtained by triturating the lyophilized samples. No activity loss was found through the triturating process, which was demonstrated by the test of activity of interferon before and after trituration (data not shown). Microparticles with different sizes were obtained by being triturated for different time and then separated through sieves with different meshes. Microparticle size was analyzed with a laser diffraction particle size analyzer.

2.2.2. Preparation of microspheres

Microspheres were prepared with three methods, i.e. solid in oil in oil (S/O/O) method, water in oil in water (W/O/W) method and solid in oil in water (S/O/W) method.

2.2.2.1. S/O/O method. S/O emulsion was obtained by putting gelatin microparticles into the first oil phase, which was composed of acetonitrile and PLGA. 80 ml cotton oil with 0.05% egg phosphatidylcholine was served as the second oil phase. The mixture of S/O emulsion and the second oil phase was homogenized for 1 min at 5000 rpm to obtain S/O/O emulsion and then stirred for 4 h at 1000 rpm. In the next step, 10 ml ligroin was added and then stirred for 30 min at 200 rpm for hardening of the microspheres. Then microspheres were collected by centrifuging at 8000 rpm for 5 min after being washed with 10 ml of ligroin in triplicate.

2.2.2.2. W/O/W method. The first water phase was obtained by dissolving IFNα-2b gelatin microparticles into glycerol. The oil phase was obtained by adding PLGA into 1 ml of methylene chloride. The first water phase and oil phase were mixed and emulsified for 1 min at 5000 rpm and then W/O emulsion was obtained. The resulting W/O emulsion was quickly poured into 15 ml 4% (w/v) PVA solution, the second water phase, and stirred for 5 min at 1000 rpm to obtain W/O/W emulsion. The resulting W/O/W emulsion was poured into 150 ml 1% (w/v) PVA solution and stirred for another 4 h at 200 rpm for hardening of the microspheres. Microspheres were collected by centrifuging at 8000 rpm for 5 min after being washed with 10 ml of distilled water in triplicate.

2.2.2.3. S/O/W method. The oil phase was obtained by dissolving PLGA or PEGT/PBT or the mixture of PLGA and PEGT/PBT into 1 ml of methylene chloride. The S/O suspension was produced by dispersing gelatin microparticles into the oil phase, and then S/O/W emulsion was obtained by pouring S/O suspension quickly into the water phase, 15 ml 4% (w/v) PVA, under stirring at 1000 rpm for 5 min. The resulting S/O/W emulsion was then poured into 150 ml 1% (w/v) PVA under stirring at 200 rpm for 4 h for hardening of the microspheres. Microspheres were collected after being washed with 10 ml of distilled water in triplicate.

2.3. Characterization of gelatin microparticles and microspheres

2.3.1. Particle size analysis

The particle size of all obtained gelatin microparticles and microspheres was analyzed with a laser diffraction particle size analyzer. To do this, gelatin microparticles were dispersed into cotton oil and microspheres were dispersed into 0.5% (w/v) sodium carboxymethyl cellulose.

2.3.2. Determination of IFNα-2b content in microspheres

To determine the content, IFNα-2b was firstly extracted from microspheres as previously described (Johnson et al., 1997). In brief, microspheres were dissolved into 250 μl of methylene chloride by vortex for 1 min and standing for 5 min, then 750 μl of acetone was added and mixed with vortex for 1 min. The mixtures were centrifuged at 6000 rpm for 5 min. The supernatant was discarded and the precipitate containing IFNα-2b was treated repeatedly for additional two times.

The final extracted IFNα-2b was dissolved in PBS (pH 7.4) and quantified by ELISA method (Ruiz et al., 2003; Santana et al., 1999). The content of IFNα-2b in microspheres is proposed as "IFNα-2b encapsulation efficiency" which indicates the percentage of IFNα-2b encapsulated in the microspheres with respect to the total amount of IFNα-2b used in the process.

2.3.3. Determination of IFNα-2b bioactivity

The bioactivity of IFNα-2b was measured using the antiviral activity (cytopathic effect reduction) assay. IFNα-2b antiviral activity was assayed by inhibition of the cytopathic effect produced by the vesicular stomatitis virus on WISH cells (Sebeka et al., 2001).

2.3.4. In vitro release of IFNα-2b from microspheres

All microspheres used in in vitro release were sterilized by ^{60}Co radiation, a common method used in sterilization of microspheres. Although exposure to ^{60}Co radiation at an extent of 1 million rad lead to about 10% activity of interferon lost in this study, there was no effect on the release.

About 30 mg microspheres with IFNα-2b were incubated in 2 ml sterile release medium, which consisted of PBS (50 mM, pH 7.4), Tween 20 (0.02%, w/v) and Tween 80 (0.2%, w/v). The above suspension was incubated in a water-bath incubator at 37 °C (±2 °C). At each sampling time, the samples were centrifuged and the supernatant was withdrawn and replaced with the same volume of fresh

medium. The amount of IFNα-2b released was estimated by ELISA assay.

2.3.5. In vivo release of IFNα-2b from microspheres

2.3.5.1. Animals. Twelve Sprague–Dawley rats weighing between 165 and 195 g were used. All animal experiments complied with the requirements of the National Act on the use of experimental animals (People's Republic of China) and were conducted with the approval of the ethical committee for use of laboratory animals at our institute.

2.3.5.2. In vivo release study. Microspheres containing 200,000 IU of IFNα-2b were suspended in 1 ml sterile solution of sodium carboxymethyl cellulose (0.5%, w/v) and mannitol (5%, w/v). Six rats received 1 ml of microsphere suspension and another six rats received 0.2 ml of IFNα-2b solution as control. Plasma samples were collected from the animals at different time points, stored in frozen form and analyzed for anti-virus activity of IFNα-2b.

3. Results and discussion

Protein delivery from microspheres is very promising for avoiding proteolysis and sustaining protein release. However, complete protein release with a zero-order profile still cannot be achieved (Lee et al., 2007; Kim et al., 2006), which is mainly due to the protein instability problems occurring in the release period. For example, during the incubation stage, moisture-induced aggregation and interaction with polymer would limit protein release. As the results, incomplete release subsequent to the initial burst, even after the polymer has been substantially degraded, takes place.

Isolating proteins from their microenvironment is favorable to stabilize entrapped proteins and to improve release profile. Viscosity around the protein has been successfully enhanced with this aim in mind. In this study, we focused on maintaining protein stability during protein encapsulation and protein release. Solid protein pre-lyophilized with gelatin and zinc salt was incorporated into organic phase without influence of organic solvent and violent stir. Gelatin, which could formulate an internal highly viscous solution after hydration, was used to protect proteins during both encapsulation process and release period from adsorption, aggregation, degradation and reduce the burst release. In addition, zinc may also have a favorable influence on protein stability and minimizing initial burst (Jones et al., 1997; Bernstein et al., 2002).

3.1. Effect of preparative method on microspheres

Microspheres were prepared in various methods basing on three main strategies: emulsion method (solvent extraction/evaporation), coacervation (phase separation) and spray-drying. Among all these methods, emulsion method is the most commonly used one, for example water-in-oil (W/O) (Jorgensen et al., 2006), water-in-oil-in-water (W/O/W) (Coombes et al., 1998; Freitas et al., 2005; Takada et al., 2003), water-in-oil-in-oil (W/O/O) (Lu and Park, 1995a) and O/W (Park et al., 1998). There are water/oil interfaces existing during preparation by these methods, which lead to protein aggregation and inactivation (van de Weert et al., 2000). So solid-in-oil-in-water (S/O/W) (Morita et al., 2000), Solid-in-oil-in-oil (S/O/O) (Carrasquillo et al., 2001), solid-in-oil-in-oil-in-water (S/O/O/W) (Yuan et al., 2009) and solid-in-water-in-oil-in-water (S/W/O/W) (Lee et al., 2007) were used for the activity and controlled release of proteins.

In this paper, S/O/O, S/O/W and W/O/W methods were used to prepare microspheres containing IFNα-2b. Particle sizes and encapsulation efficiencies of microspheres were 74.52 μm and 35.75% by S/O/O method, 40.40 μm and 30.77% by W/O/W method and 35.40 μm and 81.41% by S/O/W method, respectively. Higher

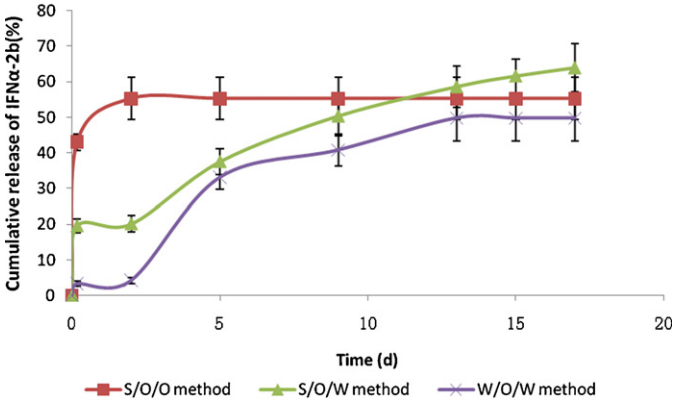

Fig. 1. Release profiles of microspheres prepared by S/O/O, S/O/W and W/O/W methods in vitro. Microspheres prepared by S/O/O, S/O/W and W/O/W methods were incubated in sterile medium at 37 °C (±2 °C) with continuous agitation. The amount of released IFNα-2b was estimated by ELISA assay. The *x*-axis is the sampling time, and the *y*-axis is the cumulative release of IFNα-2b. The release profile of microspheres by S/O/O method is indicated with (■), the release profile of microspheres by S/O/W method is indicated with (▲) and the release profile of microspheres by W/O/W method is indicated with (×).

encapsulation efficiency was found in microspheres by S/O/W method comparing to the other two methods. As shown in Fig. 1, significantly different in vitro release profiles were found in microspheres prepared by three methods. For microspheres by S/O/O method, a large burst release above 40% was observed followed by almost no further protein release. Different to S/O/O method, a less burst release of 5% with a sequential release of less than 50% was shown from microspheres by W/O/W method. The best release profile was found in microspheres by S/O/W method, which showed a burst release of less than 20% and a continuous release for 17 days with a cumulative release above 60%.

Solid-in-oil-in-oil (S/O/O) method was believed to be able to protect proteins from forming aggregates, but it also gave severe burst release and obviously incomplete release of encapsulated protein (Kim and Park, 1999; Kwon et al., 2001). In our study, S/O/O method indeed gave more distinct burst release comparing to W/O/W and S/O/W methods. The incomplete release was also observed in this paper. Lower encapsulation and cumulative release of IFNα-2b in microspheres by W/O/W method were observed, which was believed to be caused by proteins' denaturation. IFNα-2b become more sensitive to the external environment when existing in solution, so violent stir, water–oil interface, hydrophobic surface, high temperature and other factors during preparation will result in absorption, unfold, aggregation or undesired changes and lead to the loss of biological activity. As the result, low encapsulation efficiency and incomplete release will be observed. For microspheres by S/O/W method, the low burst release and large cumulative release were probably due to the use of solidified IFNα-2b during preparation and existence of gelatin and other protective agents.

3.2. Effect of gelatin microparticle size on microspheres

Microsphere size is considered important because it may affect drug encapsulation efficiency, product syringeability and the rate of drug release. Size of microspheres is mainly determined by droplet formation step. In analogy, entrapment of solid particles will influence the size of microspheres (Al-Azzam et al., 2002), furthermore, the size of microspheres increases along with the increase of encapsulated particle size.

In this study, the effects of gelatin microparticle size on microspheres were also evaluated. The drug loadings of all microparticles used here had been determined and were all

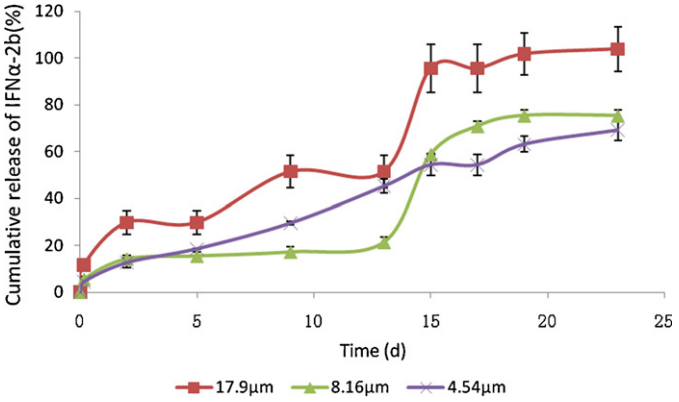

Fig. 2. Release profiles of microspheres prepared with different gelatin microparticles in vitro. The *x*-axis is the sampling time, and the *y*-axis is the cumulative release of IFNα-2b. The release profile of microspheres with gelatin microparticles of 17.90 μm is indicated with (■), the release profile of microspheres with gelatin microparticles of 8.16 μm is indicated with (▲) and the release profile of microspheres with gelatin microparticles of 4.54 μm is indicated with (×).

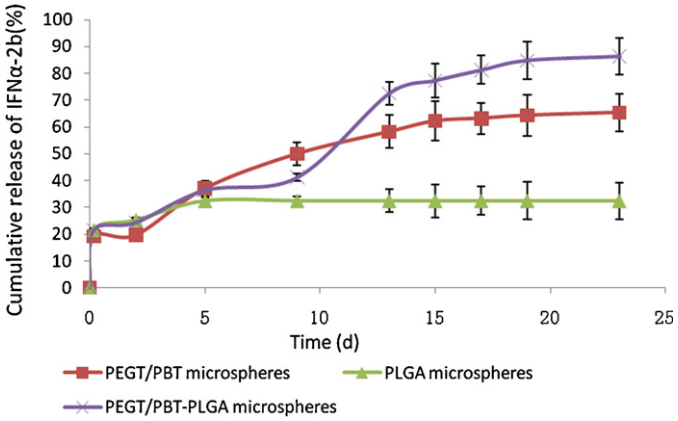

Fig. 3. Release profiles of microspheres prepared with different matrix materials in vitro. The *x*-axis is the sampling time, and the *y*-axis is the cumulative release of IFNα-2b. The release profile of microspheres with PLGA as matrix material is indicated with (▲), the release profile of microspheres with PEGT/PBT as matrix material is indicated with (■) and the release profile of microspheres with PEGT/PBT-PLGA (9:1, w/w) as matrix material is indicated with (×).

about 3,000,000 IU/ml. Microspheres were prepared with gelatin microparticles of 17.90 μm, 8.16 μm and 4.54 μm, respetively, and the corresponding sizes and encapsulation efficiencies were 33.01 μm and 79.31%, 28.60 μm and 88.68%, 23.27 μm and 93.45%, respectively. With the increase of gelatin microparticle size, decrease of encapsulation efficiency accompanied with obvious burst release and time-lag was observed (Fig. 2). The results are logistic because it is obviously difficult for large particles to be encapsulated and distribute uniformly in microspheres. In addition, large microparticles near or at the surface of microspheres will also lead to severe burst release without further protein released since there is no microparticles or proteins existing around. On the contrary, small microparticles will be encapsulated easily and distribute uniformly in microspheres, and as a result, protein will be released more steadily and slowly.

3.3. *Effect of matrixes on microspheres*

Extensive investigations have been carried out on polymers for controlled release system of peptides and proteins. PLGA is considered one of promising polymers valid for preparation of protein delivery microspheres. However, much concern is also expressed about the effects of PLGA on protein stability and incomplete release (Castellanos et al., 2001; Lu and Park, 1995b; Uchida et al., 1996; Crotts and Park, 1997), which severely hamper the development of successful delivery of proteins from PLGA microparticles. The unsuccessful release from PLGA microspheres is partly due to the hydrophobic nature of the polymer (Lam et al., 2000; Blanco-Príeto et al., 2004). Hydrophilic polymers will be more promising. Recently, a series of PEGT/PBT copolymers have been studied as matrixes for controlled drug delivery (Bezemer et al., 2000; Sohier et al., 2003, 2006).

In our study, significant effect of matrixes on in vitro release, but not the particle size and encapsulation efficiency, was observed. As shown in Fig. 3, highest burst release followed by almost no further drug release from microspheres with PLGA was demonstrated. A lower burst release, a time lag and then sustained release for about 23 days with a cumulative release about 65% were found from microspheres with PEGT/PBT. Lower burst release, no time-lag and more complete release (above 80%) were shown in microspheres with PLGA and PEGT/PBT comparing to microspheres with PLGA or PEGT/PBT alone.

PLGA is generally used for preparing microspheres according to its good biodegradability and biocompatibility. So it is not surprising to find several kinds of microspheres with this matrix in clinic. However, in our study, almost no drug release sequential after a distinct burst release from microspheres made of PLGA was found. It was probably due to the adsorption and instability of protein drugs. Moisture-induced aggregation and ionic interactions are supposed to occur in the initial phase of microsphere hydration. Later, during polymer erosion, non-specific protein adsorption onto the surface of degrading polymer and covalent/noncovalent aggregation caused by acidic PLGA degradation products are reported as factors responsible for incomplete release. All of these, the hydrophobic nature of the polymer and acidic degradation products are critical factors affecting protein release kinetics from PLGA microspheres, and polymers, which are more hydrophilic and produce no acidic degradation substances, are perhaps more compatible with proteins.

PEGT/PBTs are reported well-tolerated and do not cause adverse tissue or systemic effects. These copolymers have good hydrophilicity and there is no acid substances produced when degrading. All of these characters suggest that PEGT/PBTs might be good matrix materials for sustained release system encapsulating proteins. In this paper, PEGT/PBT was also shown better than PLGA for sustained release of IFNα-2b from microspheres. IFNα-2b in microspheres made of PEGT/PBT was released continuously for 23 days without massive burst release or obvious time lag. However, for the purpose of complete release of IFNα-2b, ideal result was still not shown for PEGT/PBT. Only 65% of proteins were released from microspheres made of PEGT/PBT at 23th day. This is perhaps due to the low degradation rate of PEGT/PBT (van Dijkhuizen-Radersma et al., 2002).

Blending two polymers allows the manipulation of the timing associated with drug release (Freiberg and Zhu, 2004; Tuncay et al., 2000). So the mixture of PLGA and PEGT/PBT (9:1, w/w) was used as microsphere matrix in this paper to optimize release profile. As expected, a more satisfactory release profile was obtained from these microspheres. Addition of PLGA decreased slightly the burst release and increased the cumulative release of proteins. The decrease of burst release is perhaps caused by the minor increase of the viscosity of oil phase, which hampered the diffusion of proteins into the surface of microspheres. The increase of cumulative release might be ascribed to the low glass-transition temperature and fast degradation rate of PLGA, which lead to the formation of microchannels in the microspheres and resulted in the more complete release.

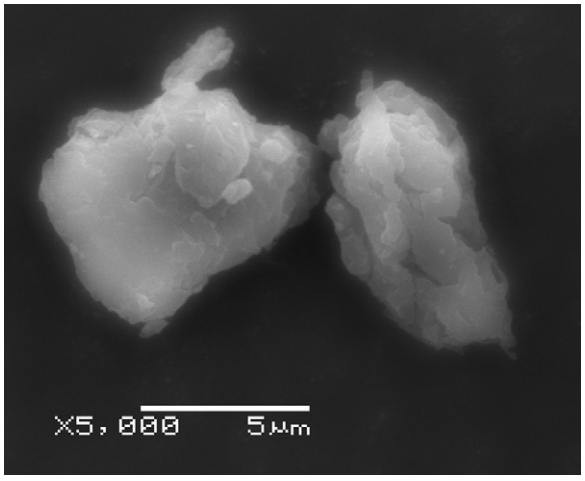

Fig. 4. Scanning electron micrograph of gelatin mciroparticle surface morphologies. The bar represents 5 μm.

3.4. Characteristics of optimal microspheres

After optimization, microspheres were prepared by S/O/W method with irregular gelatin microparticles about 5 μm (Fig. 4) and PEGT/PBT-PLGA (9:1, w/w) as matrix materials. The microspheres were round as shown in Fig. 5 and the diameter of microspheres was 28.94 μm, the encapsulation efficiency was 86.01%, the burst release was 16.69% and the cumulative release was 83.06% at 23th day. As shown in Fig. 6, an ideal zero-order release profile was obtained. In summary, IFNα-2b microspheres prepared in this paper have proper particle size, high encapsulation efficiency, sustained and complete release for 23 days in vitro.

3.5. In vivo release in rats

Only optimized microspheres and IFNα-2b solution were selected for in vivo release study. As shown in Fig. 7, IFNα-2b concentration in plasma raised to the maximum concentration (C_{max}) of 107.33 IU/ml at 4 h (t_{max}), and decreased to about 11 IU/ml at 1 day, then maintained at this level for about 12 days. While in Fig. 8, IFNα-2b concentration in plasma raised to the maximum concentration (C_{max}) of 182.59 IU/ml at 1 h (t_{max}) and decreased quickly to about 3 IU/ml at 12 h. So, IFNα-2b microspheres in this paper can provide sustained and steady plasma levels of active IFNα-2b for about 13 days with a single subcutaneous injection, which is faster than what we observed in vitro.

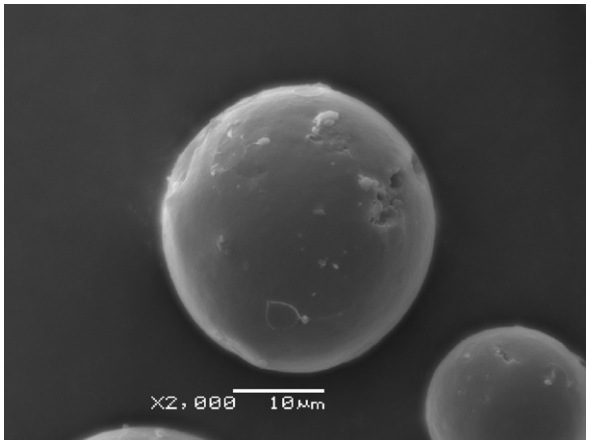

Fig. 5. Scanning electron micrograph of PEGT/PBT-PLGA (weight ratio 9:1) microsphere surface morphologies. The bar represents 10 μm.

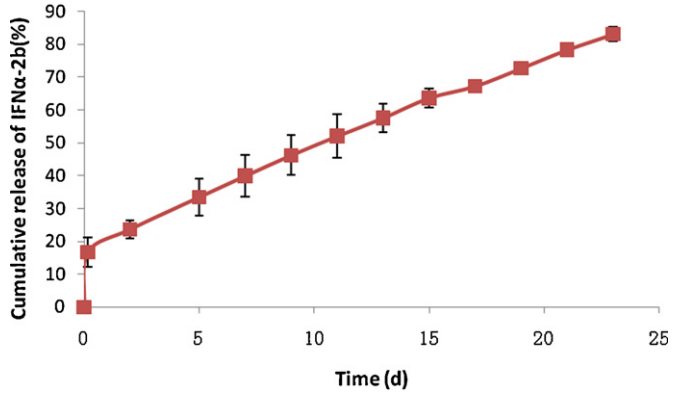

Fig. 6. Release profile of the optimal IFNα-2b microspheres in vitro. In vitro release profile of the optimal IFNα-2b microspheres is plotted with the *x*-axis as the sampling time and the *y*-axis as the cumulative release of IFNα-2b.

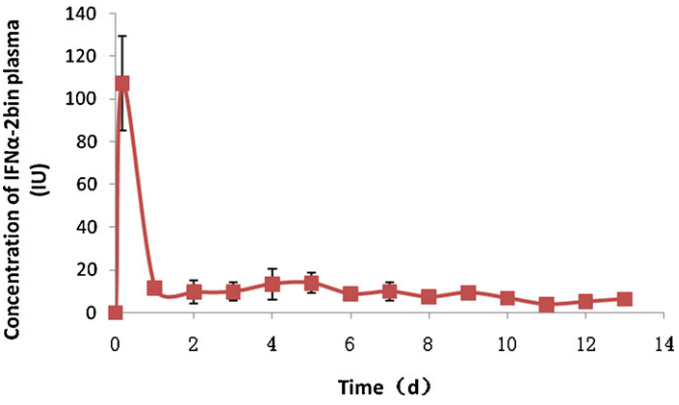

Fig. 7. Release profile of the optimal IFNα-2b microspheres in rats. Six rats were administrated with a single subcutaneous at the dose of 200,000 IU. The amount of released IFNα-2b was estimated by CPE assay. The concentration of IFNα-2b in plasma was tested with 1 day interval until 13 days. Each point represents the mean of IFNα-2b concentrations in the plasma of six rats.

Several reasons may be responsible for the difference in in vitro and in vivo drug release. For PLGA-based release system, the release of drug was mainly controlled by the degradation of polymer matrix. So it was reasonable to observe that the release of PLGA-based system in vivo was faster than that in vitro (Machida et al., 2000; Jiang et al., 2003) because degradation of PLGA in vivo was faster than that in vitro due to the foreign body response (Spenlehauer et al., 1989; Tracy et al., 1999).

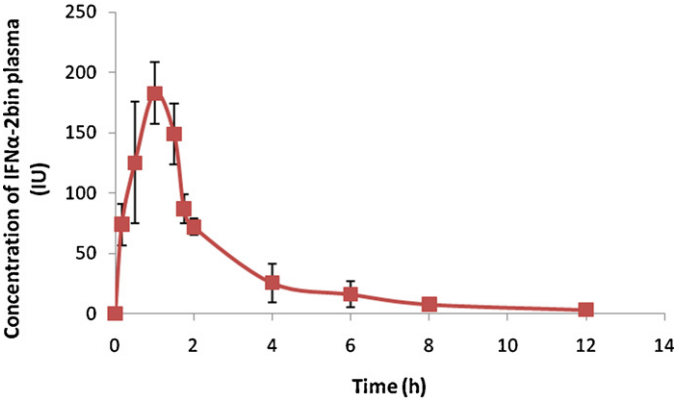

Fig. 8. Release profile of IFNα-2b solution in rats. Six rats were administrated with a single subcutaneous at the dose of 200,000 IU. The amount of IFNα-2b in plasma was estimated by CPE assay. Each point represents the mean of IFNα-2b concentrations in the plasma of six rats.

It is shown in several literatures that drug release from microspheres in animal correlates very well with drug release in human (Periti et al., 2002; Comets et al., 2000). In this study, IFNα-2b release from the microspheres in rats has shown to be fairly constant, especially in the last 12 days, so it is reasonable to anticipate that the microspheres will provide a fairly constant rate of IFNα-2b release in human and provide stable effect for above 10 days.

Acknowledgement

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