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Nanoparticles bearing polyethyleneglycol-coupled transferrin as gene carriers: preparation and in vitro evaluation

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Abstract

The aims of this work were to determine the stability of pDNA against various conditions during microencapsulation, prepare transferrin (TF)-conjugated PEGylated polycyanoacrylate nanoparticles (TF–PEG-nanoparticles), and assess its physicochemical characteristics and in vitro targeting cells association. The open circular forms of pDNA obviously increased when pDNA was emulsified into organic solution under sonification. When pDNA solution (pH 7.0) contained 1, 3 or 5% (w/v) PVA, after sonification, average 48.2, 59.4 and 62.1% of double-supercoiled DNA (dsDNA) were preserved, respectively. When medium of pDNA was 0.9% NaCl (pH 7.0), 0.1 M $NaHCO_3$ (pH 8.0) or phosphate buffer (pH 8.0), average 53.1, 69.3 and 56.9% of dsDNA remained after sonification, respectively. Poly(aminopoly(ethylene glycol)cyanoacrylate-co-hexadecyl cyanoacrylate) (poly(H_2NPEGCA-co-HDCA)) showed a slight influence on pDNA in 0.1 M $NaHCO_3$ (pH 8.0) when its concentration increased from 0.5 to 4% (w/v). TF–PEG-nanoparticles loading pDNA were spherical in shape with size under 200 nm and entrapment efficiency 35–50%. 0.1 M $NaHCO_3$ with 3% PVA (w/v) could largely reduce the damage of pDNA during microencapsulation. TF–PEG-nanoparticles bore 1–3% of the total PEG chains conjugated to TF molecules, and exhibited the burst effect with over 30% drug release within 1 day. After the first phase, pDNA release profiles displayed a sustained release. The amount of cumulated pDNA release over 7 days was: 86.3, 81.5 and 74.4% for 1, 2 and 4% polymer nanoparticles, respectively. The degree of target K562 cell binding of TF–PEG-nanoparticles was greater than that of non-targeted PEG-nanoparticles at 4 °C. The presence of free TF decreased significantly the degree of cell binding of TF–PEG-nanoparticles, which revealed that the binding of TF–PEG-nanoparticles to K562 cells was indeed receptor specific. These results suggested that TF–PEG-nanoparticles were useful for delivery of pDNA to target cells.

Keywords: pDNA; Nanoparticles; Transferrin; Polyethylene glycol; Poly(cyanoacrylate)

1. Introduction

A major goal to be reached in gene therapy protocols is the efficient and specific delivery of therapeutic genes into the desired target cells. Although the viral-based delivery systems can efficiently introduce genes, they are encountered with immunogenity and toxicity in preliminary clinical studies (Van de Wetering et al., 1998; Tagawa et al., 2002). Non-viral delivery systems including polycations and particulate carriers have been researched and proposed as alternatives to viral vector because their potential advantages, such as relative ease of large-scale production, non-immunogenic and relative safety (Kircheis

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et al., 2001; Cemazar et al., 2002; Hasegawa et al., 2002). However, these non-viral delivery systems lack efficiency of gene transfer of viruses. Therefore, it is one of the most important challenges to find efficient and specific non-viral gene delivery systems for researchers interested in gene therapy.

The long-circulating liposomes with various ligands have been investigated to deliver therapeutic genes into the target cells (Ishida et al., 2001; Derycke and De Witte, 2002; Hofland et al., 2002; Shi et al., 2001, 2002). However, commercialized cationic liposomes have shown some disadvantages, for example, (1) they are not stable after purchase, during storage and after mixing with the DNA solution; (2) they are strongly influenced by the pH and/or osmolarity of the added DNA solution; (3) most of them are not available, except for some formulations such as Genetransfer®, for use in the presence of fetal bovine serum (FBS) due to alterations of their vesicle structures resulting from interaction with serum components (Kikuchi et al., 1998, 1999). Recently, there is an increase in interest to develop long-circulating nanoparticles as proteins/peptides and plasmid DNA (pDNA) carriers (Quellec et al., 1998; Tobío et al., 1998; Li et al., 2001a,b,c; Perez et al., 2001). This long-circulating nanoparticles compared with other long-circulating systems show better shelf stability and ability to control the release of the encapsulated drugs (Gref et al., 1994; Bazile et al., 1995). Unfortunately, only a few studies have been done for the long-circulating nanoparticles with site-specific targeting ligand used to deliver pDNA until today (Mao et al., 2001).

We are interested in designing a new delivery system for pDNA. This new concept proposed in this study is to design PEG-coated biodegradable polycyanoacrylate nanoparticles conjugated to transferrin (TF), an iron-transporting serum glycoprotein which binds to a receptor expressed at the surface of most proliferating cells with particularly high expression on erythroblasts and cancer cells (Wagner et al., 1994). Here, we describe the influences of various preparation parameters during double microencapsulation on the stability of pDNA, systematically investigate the preparation and physicochemical characteristics of PEGylated polycyanoacrylate nanoparticles with TF (TF–PEG-nanoparticles), and in vitro release of pDNA from nanoparticles and TF–PEG-nanoparticles-cell association.

2. Materials and methods

2.1. Chemicals

Cyanoacetic acid (purity, 99%), polyvinyl alcohol (PVA, $M_w = 16$ kDa, 98% hydrolyzed) and polyvinylpyrrolidone (PVP, $M_w = 40$ kDa) were obtained from Fluka (Buches, Switzerland). *n*-Hexadecanol, trifluoroacetic acid (TFA) and human TF were purchased from Sigma Chemical Co. (St. Louis, MO). pGShIL-2tet coding for human interleukin-2 (IL-2, supercoiled, 4.3 kb) was used and described in Schreiber et al. (1999). *t*-Boc-NHPEG-NHS ($M_w = 3400$) was purchased from Shearwater Polymers, Inc. (Huntsville, AL). Poly(aminopoly(ethylene glycol)-cyanoacrylate-co-hexadecyl cyanoacrylate) (poly(H_2-NPEGCA-co-HDCA) was synthesized according to the method of Stella et al. (2000).

2.2. Stability of pDNA

The stability of pDNA against various conditions during the double emulsification microencapsulation was investigated. First, the influence of organic solutions on pDNA was checked up, 0.6 ml of pDNA containing 36 μg pDNA in water (pH 7.0) was emulsified in 2 ml of organic solutions by sonification (Sonifier 250, Branson, output = 10 units) for 10 s in an ice bath. Then, the emulsion was centrifuged at 5000 × *g* for 10 min (4 °C, Eppendorf, Germany) to break emulsion and separate aqueous phase from oil phase. The pDNA was extracted from the aqueous phase and agarose gel electrophoresis (0.7% agarose gel, 110 V/cm in a Tris–acetate–EDTA buffer system (pH 8.0) for 60 min, and pDNA was visualized using ethidium bromide staining) was used for determining the integrity of DNA. Second, the protection of PVA and PVP on pDNA was investigated, 2 ml of pDNA solution (60 μg/ml) with different concentrations of PVA or PVP was homogenized by sonification (output = 10 units) for 10 s in an ice bath. Then, the relative fluorescence intensity of pDNA was determined by Pico Green® assay. Pico Green® analysis for double-supercoiled DNA (dsDNA) was performed in 96-well plates with standard fluorescein wavelengths (excitation: 480 nm and emission: 520 nm) according to the manufacture's instructions (Molecular Probes, Eugene, OR) using an automated plate reader (Tecan

Spectrafluor Plus, Austria). The lack of interference of PVA or PVP in the fluorescence developed by the Pico Green®-DNA was assessed by comparing the calibration curves performed with or without each of these agents. Third, in order to examine the influence of sonification on pDNA in various mediums, 2 ml of pDNA in various aqueous mediums (60 μg/ml) was treated by sonification (output = 10 units) for 10 s in an ice bath. Then, fluorescence intensity of pDNA was determined by Pico Green® assay. Finally, the influence of poly(H_2NPEGCA-co-HDCA) on pDNA was investigated, 0.6 ml of pDNA in 0.1 M $NaHCO_3$ solution (60 μg/ml) was emulsified in 2 ml of dichloromethane (DCM)/ethyl acetate (1:1) containing different concentrations of polymer by sonification (output = 10 units) for 10 s in an ice bath. The emulsion was centrifuged at 5000 × *g* for 10 min (4 °C, Eppendorf, Germany). The ratio of supercoiled to degraded pDNA from the aqueous phase was quantitated densitometrically using a Kodak Scanner (Kodak digital science, electrophoresis documentation and analysis system 120).

2.3. Preparation of TF–PEG-nanoparticles

Poly(H_2NPEGCA-co-HDCA) nanoparticles (PEG-nanoparticles) loading pDNA were prepared by a water–oil–water solvent evaporation techniques in advance. Briefly, 0.6 ml of pDNA in 0.1 M $NaHCO_3$ solution (60 μg/ml, pH 8.0) was emulsified in 2 ml of DCM/ethyl acetate (1:1) containing poly(H_2NPEGCA-co-HDCA) by sonification in an ice bath for 5 s (Sonifier 250, Branson, output = 10 units) to form primary emulsion (W/O). Then, the first emulsion was poured into 10 ml of the PVA aqueous solution (1.0%, w/v) and treated by sonification in an ice bath for 5 s (output = 10 units). The double emulsion (W/O/W) was diluted in 20 ml PVA solution (0.3%, w/v) under moderate magnetic stirring. The magnetic stirring was maintained for 1 h to allow solidification of the nanoparticles, and organic solution was eliminated by evaporation under reduced pressure (Rotavapor R-114, Büchi, Switzerland) at 37 °C. Finally, the nanoparticles were collected by centrifugation at 39,000 × *g* for 20 min (Rc5c, Sorvall Instruments).

TF–PEG-nanoparticles were prepared by coupling of TF to PEG-nanoparticles using periodate oxidation method. Briefly, 1 ml of TF solution (80 mg/ml) was mixed with 50 μl sodium periodate solution (20 mg/ml). The mixture was kept on ice for 90 min. The oxidized TF was purified by Sephasex G-25 PD 10 column (Pharmacia, 150 mM NaCl, 10 mM HEPES, pH 7.3). This oxidized TF (0.5 μmol) was incubated with 1 ml of PEG-nanoparticles. After 1 h at room temperature, 50 μl of 1 M glycine solution was added, and the TF–PEG-nanoparticles were purified to remove excess free TF by centrifugation at 39,000 × *g* for 20 min (Rc5c, Sorvall Instruments).

2.4. Physicochemical characteristics of TF–PEG-nanoparticles

The pDNA encapsulated in nanoparticles was determined by suspending 10 mg of nanoparticles loading 1.4–1.8 μg DNA in 1 ml of 10 mM Tris–EDTA buffer (pH 7.4), then, adding 2 ml of DCM and shaking, following centrifugation at 5000 × *g* for 10 min (Eppendorf, Germany). The amount of pDNA in the aqueous layer was calculated from the fluorescence by Pico Green® assay. The integrity of pDNA was checked up by agarose gel electrophoresis after the other portion was precipitated by the addition of EtOH.

The morphology was examined by transmission electron microscopy (TEM, CM12, Philip) following negative staining with sodium phosphotungstate solution (0.2%, w/v). Size distribution and ζ-potential of nanoparticles were measured by laser light scattering following their resuspension in 10 mM NaCl (pH 7.2) using a Zetasizer 3000 HS (Malvern Instruments, UK).

For assay of TF bound to PEG-nanoparticles, TF–PEG-nanoparticles and TF standards in HBS were bound to a nitrocellulose membrane (Schleicher & Schuell, 0.22-μm pore size). The TF content of each sample was determined by exposing the membrane for 4 h at room temperature to a mouse antibody (Chemicon MAB 033-19/1), and then exposed for 1 h to the second antibody (Alexa Fluor® 488, A-11001). Finally, the fluorescence analysis of the membrane was performed with standard fluorescein wavelengths (excitation: 495 nm and emission: 520 nm).

2.5. In vitro release experiment

Release experiment was carried out in 33 mM phosphate buffer (pH 7.4). Twenty milligram of

TF–PEG-nanoparticles was suspended in 1.5 ml of phosphate buffer preserved with 0.02% sodium azide at 37 °C under horizontal shaking (300 rpm, Thermomixer, Eppendorf, Germany). At predetermined time intervals, the suspension of nanoparticles was centrifuged at 39,000 × *g* for 20 min (Rc5c, Sorvall Instruments) and the supernatant collected for further pDNA analysis. The nanoparticles were resuspended in the same volume of fresh medium and incubated again under the same conditions. The amount of pDNA released in each time interval was determined by the Pico Green® assay.

2.6. Cell association assay

K562 cells (ATCC CCL-243) were cultured in RPMI-1640 medium with 10% fetal calf serum (FCS) and 1% L-glutamine at 37 °C in 5% CO_2 humidified atmosphere. The pDNA was labeled with the cell-impermeable fluorescent dyes YOYO-1 (Molecular Probes) with a ratio of 1 dye molecule to 300 bp. For binding experiments, 1×10^6 K562 cells were resuspended in 1 ml of RPMI 1640 medium with 10% FCS containing 5 mg of nanoparticles, and was incubated for 1 h at 4 °C. To examine the specificity of the TF–receptor-mediated binding, cells were incubated with excess dose of TF (50 μg) at 4 °C for 20 min, and then binding assays of nanoparticles were performed in medium containing excess dose of TF (50 μg) at 4 °C. Before analysis, cells were added 1 ml of ice-cold PBS, and centrifuged at 3000 × *g* for 5 min, then analysed using a FACS calibur flow cytometer (Becton Dickinson, San Jose, CA) equipped with an Argon ion laser (488 nm emission wavelength) and a diode laser (635 nm emission wavelength).

3. Results and discussion

3.1. Stability of pDNA

It is one of the most important issues that the stability of pDNA is maintained when nanoparticles are designed as carriers of pDNA. Free pDNA possesses some unique features, its supercoiled form that is more efficient in gene expression than the relaxed form is susceptible to convert to the linear and open circular forms during the microencapsulation and result in a significant reduction in gene expression (Luo et al., 1999; Wang et al., 1999). A lot of the processing parameters during microencapsulation, for example, emulsification energies, high interfacial tension at the DCM–water inter-phase, organic solution, polymer concentration and stabilizers, can influence the stability of pDNA. Our experimental results showed that sonification could obviously turn dsDNA into linear and open circular forms (Fig. 1, lane 2), which is in accordance with the reports of Capan et al. (1999). During microencapsulation of pDNA, organic solution had to be used because of hydrophobic property of polymer. The pDNA in water without any stabilizer was influenced by organic solution, for example, DCM, mixture of DCM and ethyl acetate, acetone, ether, *n*-hexane and ethynol (1:1) after sonification (Fig. 1, lanes 3–8), particularly, open circular forms of pDNA obviously increased.

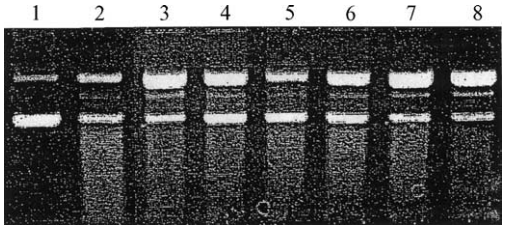

Fig. 1. Stability of pDNA under sonification (10 s) in the presence of organic solvents. Lane 1, pDNA control; lane 2, pDNA in water; lane 3, pDNA emulsified in DCM; lane 4, pDNA emulsified in DCM:ethyl acetate (1:1); lane 5, pDNA emulsified in DCM:EtOH (1:1); lane 6, pDNA emulsified in DCM:ether (1:1); lane 7, pDNA emulsified in DCM:*n*-hexane (1:1); lane 8, pDNA emulsified in DCM:acetone (1:1).

The protection of pDNA by PVA or PVP was shown in Fig. 2. When pDNA solution (pH 7.0) contained 1, 3 and 5% PVA, after sonification, average 48.2, 59.4 and 62.1% of the original fluorescence intensity of pDNA were preserved, respectively. PVP showed a weak protective effect on pDNA compared with PVA. This difference could result from properties of PVA and PVP. A great deal of foam occurred when pDNA solution with PVA was treated by sonification, which could form a layer of membrane around pDNA, therefore, indirectly reducing the damage from sonification. However, no obvious foam occurred during sonification of pDNA solution with PVP.

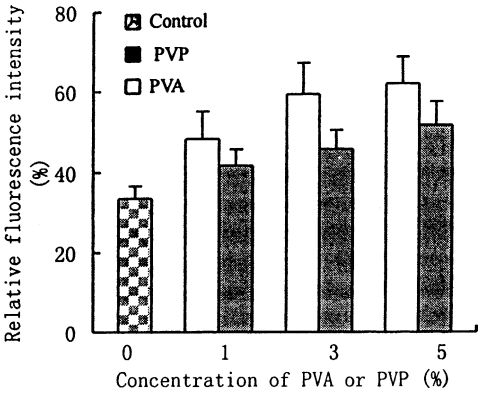

Fig. 2. Stability of pDNA under sonification in the presence of PVA and PVP. Data were given as mean ± S.D. for $n = 3$.

The influence of various mediums on stability of pDNA was showed in Fig. 3. When medium of pDNA was 0.9% NaCl (pH 7.0), 0.1 M $NaHCO_3$ (pH 8.0) or phosphate buffer (pH 8.0), after sonification, average 53.1, 69.3 and 56.9% of the original fluorescence intensity of pDNA remained, respectively. In contrast, the original fluorescence intensities of pDNA were preserved 33.5% when medium is water, or no more than 50% for PBS (pH 6.0 and 7.0). This can be explained by cations neutralizing the backbone charge more efficiently, thereby reliving the repulsive

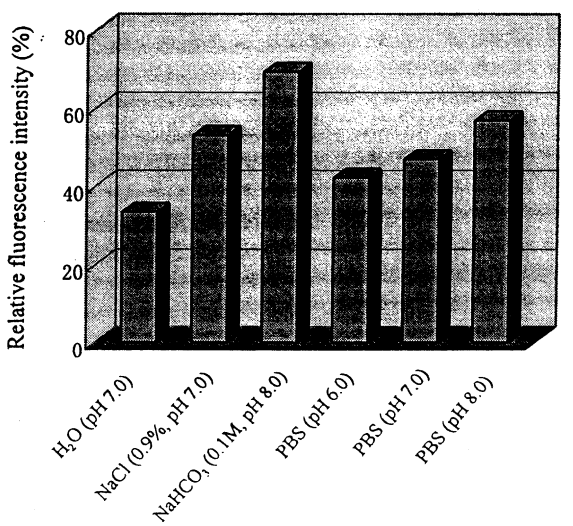

Fig. 3. Stability of pDNA in different medium under sonification. Data were given as mean ± S.D. for $n = 3$.

interaction between the negatively charged phosphodiester backbone of the paired strands (Bergstrom et al., 1998; Walter et al., 1999). In addition, it also can be relation with their pH. Two mechanisms can involve degradation of DNA at low pH: first, duplex formation of dsDNA is an equilibrium process. A heat denaturation process is widely used to separate single strands from dsDNA and is generally reversible. An increase in hydrogen ion concentration also shifts the equilibrium force from double-stranded helix to a single-stranded coil because protonation of the bases in the single strand is energetically more favorable (Bergstrom et al., 1998); Secondly, irreversible inactivation is probably to a large extent due to acidic-catalyzed depurination of single-stranded DNA and chain breaks occurring as a consequence of depurination (Lindahl and Andersson, 1972a; Lindahl and Nyberg, 1972b).

Polymer showed a slight influence on pDNA in 0.1 M $NaHCO_3$ (pH 8.0) under sonification. The ratio of supercoiled to degraded pDNA was between 61.7 ± 2.8% and 59.6.2 ± 3.4% when its concentration increased from 0.5 to 4% (w/v), which showed a slight decline compared with control (64.3 ± 1.9%). Preliminary experiment showed that drop of pH did not occur in the aqueous phase containing pDNA after emulsification and separating by centrifuge (data not shown).

3.2. Preparation and characteristics of TF–PEG-nanoparticles

The characteristics of TF–PEG-nanoparticles were summarized in Table 1. The pDNA was incorporated in TF–PEG-nanoparticles with different encapsulation efficiency by double emulsion technique. TF–PEG-nanoparticles showed a slight increase of the encapsulation efficiency with copolymer concentration, and the highest encapsulation efficiency could access about 50% when the concentration of polymer was 4% (w/v).

TF–PEG-nanoparticles observed by TEM showed spherical in shape (Fig. 4). The particle size is an important property of nanoparticles drug delivery system. The main reason is that particle size has a great effect on drug release characteristics and drug distributions in different organs of the body, particularly, tumor tissues. In addition, injectable nanoparticles can

Table 1
Physicochemical characteristics of TF–PEG-nanoparticles

Concentration of copolymer (%)	Entrapment efficiency (%)	Particle size (nm)	ζ-potential (mV)
0.5	36.4 ± 3.6	131.6 ± 6.9	−6.6 ± 1.1
1.0	40.7 ± 2.2	149.9 ± 7.5	−5.9 ± 2.7
2.0	44.6 ± 3.5	136.7 ± 6.5	−6.8 ± 2.4
4.0	49.3 ± 4.2	154.2 ± 8.8	−4.2 ± 1.3

Mean ± S.D., each batch was measured in triplicate.

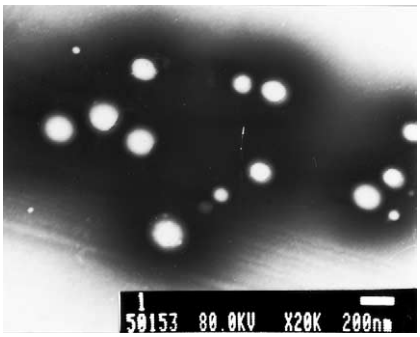

Fig. 4. Transmission electron micrographs of TF–PEG-nanoparticles.

not be too large to pass through a syringe needle. The particle size of TF–PEG-nanoparticles is no more than 200 nm. The nanoparticles loading pDNA exhibited a slightly negative surface charge (about −4.0–7.0 mV).

Fig. 5 showed that non-encapsulated pDNA was predominantly supercoiled, although a small amount of open circular DNA was visible (lane 2). The pDNA remained to a large extent dsDNA after encapsulation in nanoparticles if inter-aqueous phase was 0.1 M $NaHCO_3$ with 3% PVA (w/v, lanes 3–6). The open circular pDNA was also present after encapsulation, but a significantly smaller extent when compared with pDNA in water as inter-aqueous phase (lanes 7–8), which meant that 0.1 M $NaHCO_3$ with 3% PVA (w/v) could reduce the damage of pDNA during microencapsulation.

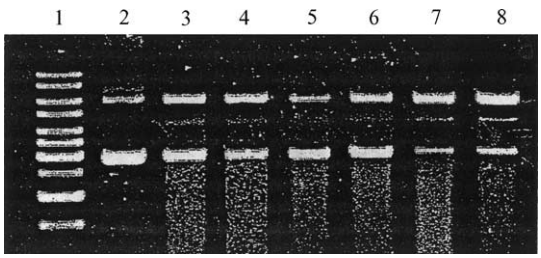

Fig. 5. Agarose (1.0%) gel electrophoresis of pDNA extracted from TF–PEG-nanoparticles. Lane 1, DNA ladder; lane 2, pDNA control; lane 3, pDNA extracted from TF–PEG-nanoparticles (0.5%, w/v); lane 4, pDNA extracted from TF–PEG-nanoparticles (1%, w/v); lane 5, pDNA extracted from TF–PEG-nanoparticles (2%, w/v); lane 6, pDNA extracted from TF–PEG-nanoparticles (4%, w/v); lanes 7 and 8, pDNA in water control, pDNA extracted from TF–PEG-nanoparticles (1%, w/v) and pDNA in water.

In order to evaluate the extent of TF conjugation in TF–PEG-nanoparticles loading pDNA, the coupling efficiency of TF was estimated by quantitative analysis that was performed after rinse of the conjugate. The average number of TF molecules per thousand PEG chains was calculated by the assumption that the molecular weight of TF is 80,000. It was found that 1–3% of the total PEG chains were linked to TF molecules.

3.3. *In vitro release experiments*

The in vitro release profiles of pDNA were obtained by representing the percentage of pDNA release with respect to the amount of pDNA encapsulation in nanoparticles (Fig. 6). The pDNA loaded nanoparticles prepared with different polymer concentrations exhibited the burst effect with over 30% drug release within 1 day. In general, the release of drugs from polymer nanoparticles mainly has two mechanisms, namely, diffusion through pores or channels formed during nanoparticle preparation, and release following polymer degradation or solubilization. The strong burst effect observed for pDNA loaded nanoparticles was probably due to the diffusion of surface localized pDNA. For three nanoparticles loading pDNA, the difference of pDNA release in the first phase could be from different amounts adsorbed onto the wall of

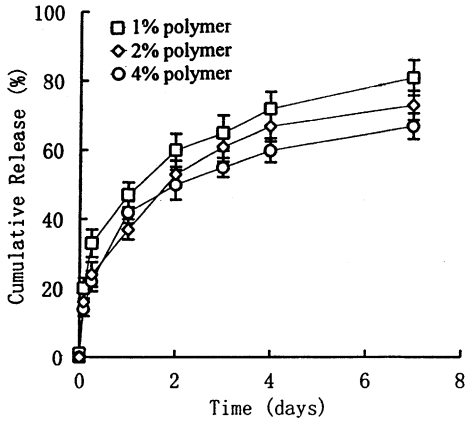

Fig. 6. Release of pDNA from TF–PEG-nanoparticles. Data were given as mean ± S.D. for $n = 3$.

nanoparticles that would be immediately released during the initial stage. To TF–PEG-nanoparticles, after 1 day, pDNA release profiles displayed a sustained release. The amount of cumulated pDNA release over 7 days was: 81.3, 73.5 and 67.1% for 1, 2 and 4% polymer nanoparticles, respectively. This sustained release could result from diffusion of pDNA through polymer pores or channel as well as the erosion and degradation of the polymer.

3.4. Cells association assay

It has been known that many ligand/receptor pairs after endocytosis are targeted to lysosomes and degraded by hydrolytic enzymes, but the receptor-mediated endocytosis process of TF is different from them in several respects, for example, after binding of TF to the receptors on the cells surface, the TF–receptor complexes are internalized to form endosomes through clathrin-coated vesicles. After internalization, iron-loaded TF releases its iron at low endosomal pH, whereas iron-free TF-remains bound to the receptor. These complexes are sorted into exocytic vesicles for delivery back to the cell surface and iron-free TF is released. The entire TF cycle takes only 4–5 min with a mean transit time of about 10 min, and avoids the lysosomal compartment (Klausner et al., 1983; Bali et al., 1991; Wagner et al., 1994). In this work, we selected TF as ligand to prepare TF–PEG-nanoparticles, which

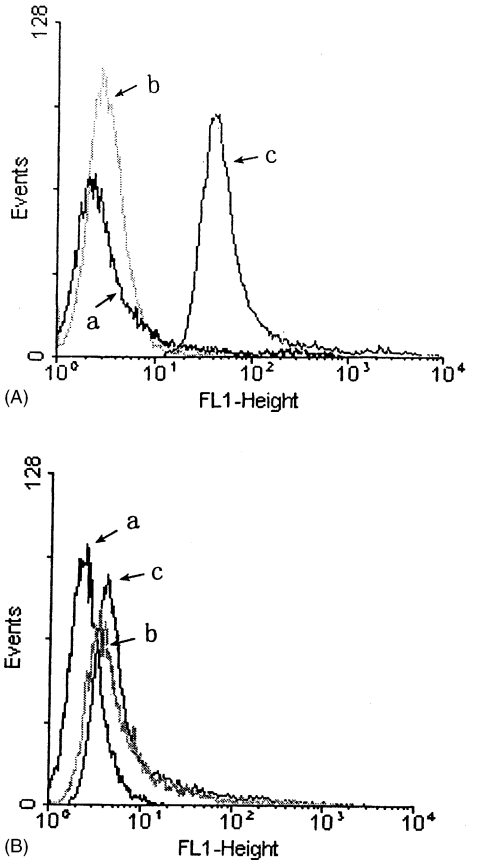

Fig. 7. Flow cytometry of K562 cells incubated with PEG-nanoparticles and TF–PEG-nanoparticles at 4 °C: (A) without free TF; (B) with free TF; line a: cells control; line b: PEG-nanoparticles; line c: TF–PEG-nanoparticles.

could avoid problem of immunological incompatibility for the cytoplasmic delivery of genes because TF is an isologous protein. The data at 4 °C (no internalization) showed that the degree of target cell binding of TF–PEG-nanoparticles was greater than that of non-targeted PEG-nanoparticles (Fig. 7A). The presence of free TF decreased significantly the degree of cell binding of TF–PEG-nanoparticles, and the fluorescence of TF–PEG-nanoparticles showed a negligible difference from that of PEG-nanoparticles (Fig. 7B). These results revealed that the binding of TF–PEG-nanoparticles to K562 cells was indeed receptor specific. It would be valuable to know the in vivo lifetime of TF–PEG-nanoparticles on target cells. However, the mode of intracellular delivery and

the fate of the TF–PEG-nanoparticles need still be investigated further.

In conclusion, 0.1 M $NaHCO_3$ with 3% PVA (w/v) could largely reduce the damage of pDNA during microencapsulation. TF–PEG-nanoparticles loading pDNA were spherical in shape with entrapment efficiency 35–50%. The in vitro release experiment showed that over 30% of drug released within 1 day. After the first phase, pDNA release profiles displayed a sustained release. The amount of cumulated pDNA release over 7 days was: 86.3, 81.5 and 74.4% for 1, 2 and 4% polymer nanoparticles, respectively. TF–PEG-nanoparticles bearing 1–3% of the total PEG chains conjugated to TF molecules, and exhibited that the degree of target K562 cell binding of TF–PEG-nanoparticles was greater than that of non-targeted PEG-nanoparticles at 4 °C. The presence of free TF decreased significantly the degree of cell binding of TF–PEG-nanoparticles. These results suggested that TF–PEG-nanoparticles were useful for delivery of pDNA to target cells. Further studies would focus on optimization of entrapment efficiency, and would evaluate the in vitro and in vivo transfection efficiency of this delivery system.

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