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Non-viral gene delivery carrier of probe type host molecule——Interactions between DNA and β-cyclodextrin derivative complexes (I)

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Abstract A host type non-virus gene delivery carrier, phenanthroline-β-cyclodextrin derivative host molecule, was produced which can be used as molecular probe. Interactions between DZY-1 and DNA were investigated by electrophoresis assay. *Hin*d III enzyme inhibition assay was carried out using DNA condensates induced by host molecules or host-guest molecule complexes to explore their ability to inhibit enzyme digestion. Micro-structure of DNA condensates induced by host molecules and host-guest molecule complexes was observed by scanning electron microscope (SEM). Our work indicates the delivery mechanism of DZY-1 used as a gene delivery carrier and also provides a method to design and produce non-virus gene delivery carriers.

Keywords: phenanthroline-β-cyclodextrin derivatives (DZY-1), λ-DNA, non-virus gene delivery carrier, agarose gel electrophoresis, scanning electron microscope (SEM).

Gene therapy is a common process to deliver extrinsic genes into target cells for further gene expression, for the purpose of treating diseases. A well designed gene delivery carrier can efficiently package and protect nucleic acid from being digested by a variety of enzymes *in vivo* and should be able to specifically locate extrinsic genes into the target organisms. So how to design efficient and safe gene delivery carriers became the most challenging problem in cancer therapy. Carriers are the central technique in gene therapy research, which are mainly divided into two types: virus carriers and non-virus carriers. Virus carriers include retrovirus, adenovirus, herpes simplex virus, and lentivirus[1,2], which have the advantage of low pathogenic rate and high transfer rate. But as a kind of modified virus, this kind of carriers is very expensive and can induce severe immunoreactions and even cancer. This disadvantage greatly inhibits its application in clinic as a gene carrier. Non-virus gene delivery carriers, such as cationic diblock copolymer vesicles[3,4], polylysine peptides[5,6] and cationic lipids[7,8], have the advantage of low price, convenient operation, high efficiency and no immunogenicity[9−11]. They have become the most promising substitutions of virus gene therapy carriers.

β-cyclodextrin (β-CD) is circle olig-glucose. The hydrophobic cave in its column structure can combine with many molecules to form host-guest type super molecule complexes through weak interactions. Recently, designing and producing new cyclodextrin derivatives with functional groups as host molecules in super-molecule complexes, probing into the recognition effect between host and guest molecules, constructing molecule assembly in nano-scale, and investigating the characteristics of functional groups are always the hot points of chemistry, biology and pharmacy researches[12,13]. In this study, we used newly synthesized phenanthroline-β-cyclodextrin derivatives (DZY-1) as host probing molecules, investigated its interactions with DNA and illustrated its mechanism as a gene delivery carrier. Our work provides a new method to design and produce non-virus gene delivery carriers.

1 Experimental

1.1 Materials

β-cyclodextrin was obtained from Shanghai Reagent Factory, λ-DNA, and *Hin*d III were purchased from TaKaRa Biotechnology (Dalian) Co. Ltd. 1-adamantanamine hydrochloride (AM) was purchased from ACROS Organics. TE buffer (pH 8.0) 10 mmol/L Tris-HCl (pH 8.0), and 1 mmol/L EDTA. TBE electrophoresis buffer 89 mmol/L Tris-boric acid, and 2 nmol/L EDTA. Electrophoresis system Sub-Cell GT POWER/PAC3000 was purchased from BIO-RAD, and X-650-scanning electron microscope was purchased from Hitachi Ltd. The water is remove-ionic-distilled water. All the reagents are of the best value and analytical reagent.

1.2 Synthesis of phenanthroline-β-cyclodextrin derivatives (DZY-1)[7]

(i) Synthesis of 2-(4′-hydroxidebenzene)-imidazole-[5,6-f]1,10-phenanthroline (HOP). First o-phenanthroline was treated with nitric acid to make 1, 10-phenanthroline-5,6-ketone. Then 10-phenanthroline-5,6-ketone was refluxed with 4-hydroxybenzaldehyde in ammonium acetate and glacial acetic acid solutions for 1 h to produce HOP.

(ii) Synthesis of mono-[6-O-(p-tolylsulfonyl)]-β-cyclodextrin (6-OTs-b-CD). β-cyclodextrin in NaOH solution was treated with 4-toluene sulfonyl chloride in acetonitrile solution to make 6-OTs-β-CD.

(iii) HOP and 6-OTs-β-CD were incubated in DMF) (20 mL) solution for 24 h at 80℃ to make DZY-1. The synthesizing process is shown in Fig. 1.

1.3 Investigate the interactions between the host type non-virus gene delivery carriers (DZY-1)

Preparing 0.06 μg/μL λ-DNA solution and 3 mg/mL DZY-1 solution using TE buffer. Mixing λ-DNA solution with DZY-1 solution at designed ratio in TE buffer for 30 min at 37℃. Mixing a certain amount of sample solution with loading buffer. Electrophoresis was carried out using 0.5% agarose in 80 V for 100 min. The result of electrophoresis was observed under ultra-violet light.

1.4 Host-guest complex (DZY-1/AM) interacting with λ-DNA

Preparing 0.06 μg/μL λ-DNA solution, 0.338 mg/mL 1-adamantanamine hydrochloride (AM) solution and 3 mg/mL DZY-1 solution using TE buffer. Mixing host and guest molecules at 1∶1 ratio ∶ mixing 0.338 mg/mL adamantanamine hydrochloride solution and 3 mg/mL DZY-1 solution at mole ratio 1∶1 and keeping the mixture at room temperature for 1 h. Combining the mixture of DZY-1/adamantanamine hydrochloride with λ-DNA (TE solution) at 1∶1 and incubating the mixture at 37℃ for 30 min. After used method of 1.3 for mixing with electrophoresis.

1.5 DNA condensation induced by host and host-guest molecules and its effect to inhibit Hind III digestion

Preparing λ-DNA/DZY-1 and λ-DNA/(DZY-1/AM) sample solutions according to sections 1.3 and 1.4. Mixing 20 μL sample solutions with 2 μL *Hin*d Ⅲ (15 U/L), 4 μL 10×M buffer and 14 μL distilled water to make sure the total volume of liquid is up to 40 μL. Then incubating the solution at 37℃ for 1 h. After used method of 1.3 for mixing with electrophoresis.

1.6 Scanning electron microscope observation

Preparing λ-DNA/DZY-1, and λ-DNA/(DZY-1/AM) sample solutions according to sections 1.3 and 1.4. Dipping 10 μL of two samples onto 2 cm^2 mica surface, and then the mica surface was dried with nitrogen. After being electroplated with gold, the samples were observed under scanning electron microscope.

2 Result and discussion

2.1 Interaction between host or host-guest gene delivery carrier and λ-DNA

Interactions between λ-DNA and DZY-1, λ-DNA

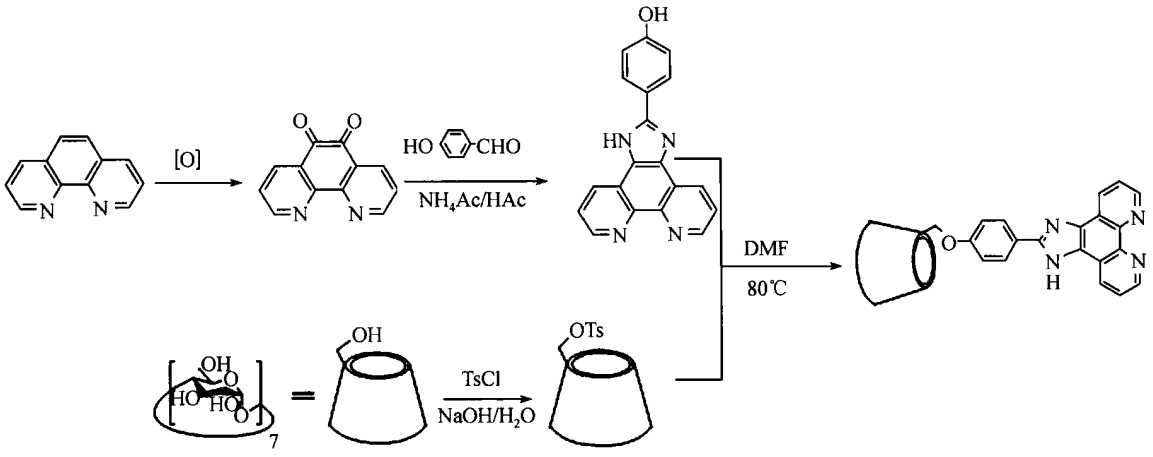

Fig. 1. Synthesizing process and structures of the host type non-virus gene delivery carriers.

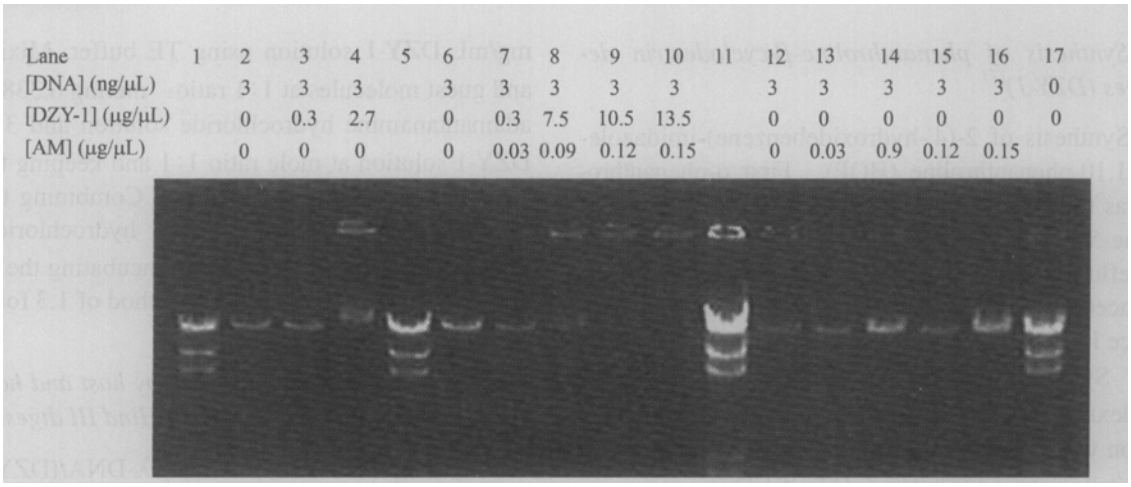

Fig. 2. Interactions between λ-DNA and DZY-1.

and (DZY-1/AM), and λ-DNA and AM were characterized by electrophoresis assay (Fig. 2). Based on the image of Fig. 2, we conclude that: 1) In λ-DNA/DZY-1 system (lanes 2－4), when the concentration of DZY-1 increased to a certain degree，the DNA lane was delayed, because DNA condensation was induced so that the molecular weight of DNA condensates increased and large complexes of DZY-1/λ-DNA were formed (lane 4). 2) In λ-DNA/(DZY-1/AM) system (lanes 6－10), electrophoresis result also shows that in relatively low (DZY-1/AM) concentration, DNA condensation could be induced and large super-molecule complexes of DNA/(DZY-1/AM) formed; in relatively high (DZY-1/AM) concentration, the complex was so large that some DNA samples were still in the loading holes (lanes 8－10), which shows that guest molecule-AM enhanced the interaction between DZY-1 and λ-DNA. 3) In λ-DNA/AM system (lanes 12－16), the lanes were almost in an aclinic line.

The image of electrophoresis shows that in λ-DNA/DZY-1 and λ-DNA/(DZY-1/AM) systems, super molecule complexes were formed because of interactions between DZY-1 and λ-DNA, and guest molecules can enhance such interactions, however guest molecules alone can not induce DNA condensation. The result indicates that DZY-1 has the potential to become a host type non-virus gene delivery carrier, which not only can interact with DNA to form complexes of condensate but also can cooperate with its guest molecules to induce DNA condensate and form super molecule complexes.

To further research the mechanism of DNA condensation induced by DZY-1, the authors used Argus Lab software to simulation of their interaction and combination model in Fig. 3. Fig. 3(a) is the ball-and-stick model of interaction between DZY-1 with DNA; Fig. 3(b) is interaction between ball-and-stick model of DZY-1 and ball-heap model of DNA.

From the simulation result we conclude that the mechanism of DNA condensation in λ-DNA/DZY-1 system is mainly because the probe molecules of DZY-1 (HOP) insert into DNA double helix and induce DNA condensation and form DZY-1/λ-DNA complexes. However, in λ-DNA/（DZY-1/AM） system, when guest molecules—AM were mixed with DZY-1 at ratio of 1∶1, AM insert into the hydrophobic cavities of host molecules, which will fully expose the probing molecules of DZY-1, enhance the interactions between probing molecules and DNA and finally promote condensation of DNA. So we conclude that DZY-1 is a host type gene delivery carrier with probing molecules. Further molecule dynamic research about this is processing in our lab.

2.2 Host or host-guest type gene delivery carrier inducing DNA condensation and its effect to inhibit Hind III digestion

To further probe the stability of DNA condensates induced by DZY-1, we used *Hin*d III, which can digest λ-DNA, to investigate the ability of DNA condensate in λ-DNA/DZY-1 system to inhibit the digestion of *Hin*d III. The result is shown in Fig. 4.

Based on Fig. 4, we conclude that: 1) in λ-DNA/DZY-1 system (lanes 1－3), with the concentration of DZY-1 increasing, the corresponding DNA bands

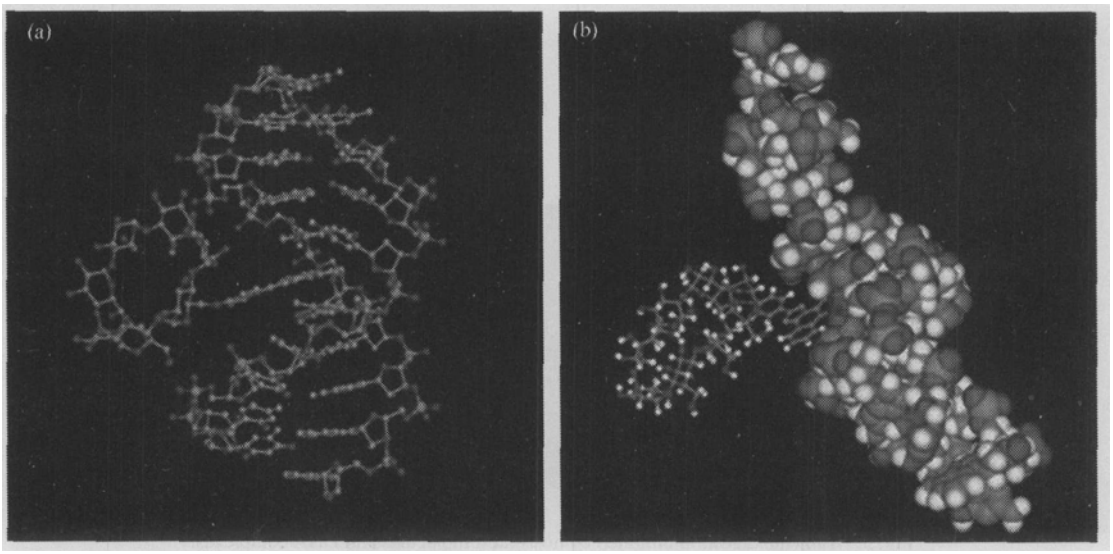

Fig. 3. Interaction of DZY-1 with DNA model simulation. (a) Ball-and-stick model; (b) ball-and-stick model of DZY-1 with ball heap model of DNA.

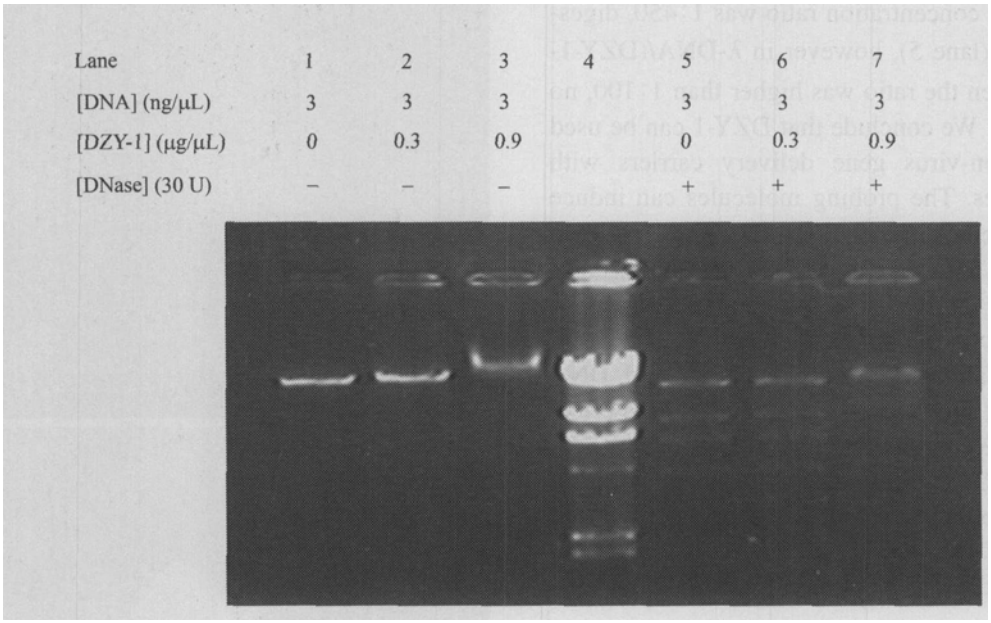

Fig. 4. λ-DNA/DZY-1 super-molecule complex inhibiting *Hin*d III digestion.

showed more trend of lagging. This is because when DZY-1 inserts into DNA structure, DNA molecules will be condensed into DNA/DZY-1 complexes with large molecule weight. 2) Corresponding *Hin*d III digestion experiment was performed in lanes 5−7. When the sample had no DZY-1, DNA was digested into 4 bands of different molecule weights (lane 5). When the concentration of DZY-1 increased, the digested bands decreased and even disappeared, which indicated that with the increasing of DZY-1, DNA condensation was enhanced and the condensates inhibited *Hin*d III digestion. Our result shows that on one hand, the probing molecule in DZY-1 can induce DNA condensation, on the other hand, DZY-1 can protect extrinsic gene from enzyme digestion during gene delivery and thereby promote gene delivery efficiency.

We further investigated the *Hin*d III inhibition effect of super-molecule complexes induced by host-guest type gene delivery carriers. Its electrophoresis image is shown in Fig. 5.

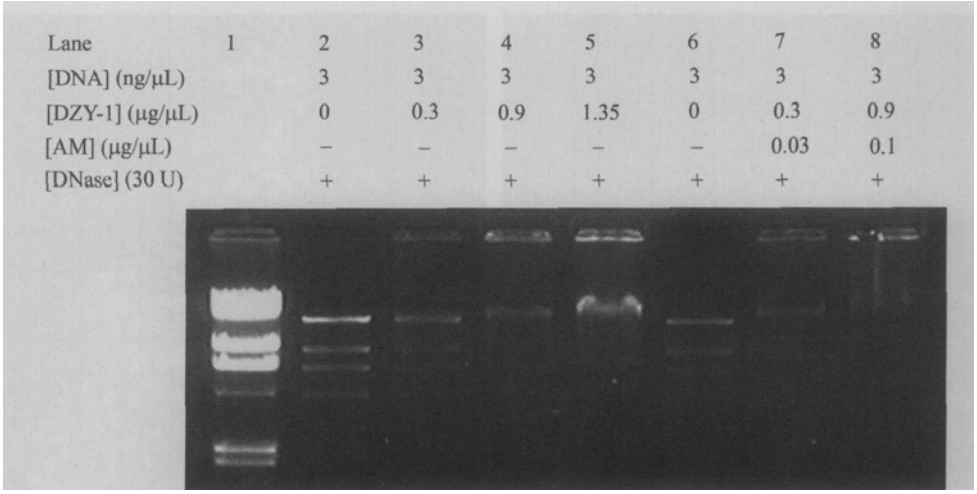

Fig. 5. Enzyme digestion inhibited by host-guest molecules and DNA complexes.

Based on Fig. 5, we conclude that in λ-DNA/DZY-1 system, when the concentration ratio was 1∶450, digestion still existed (lane 5), however in λ-DNA/(DZY-1-AM) system, when the ratio was higher than 1∶100, no digestion existed. We conclude that DZY-1 can be used as host type non-virus gene delivery carriers with probing molecules. The probing molecules can induce extrinsic DNA condensation and the β-cyclodextrin structure in host molecules can carry guest molecules (drugs or active components) and combine DNA to form super-molecule complexes. Guest molecules enhanced the formation of complexes, promoted DNA condensation and finally enhanced the inhibition effect of super-molecule complexes to *Hin*d III enzyme. DNA condensation induced by certain concentration of DZY-1 can inhibit enzyme digestion. So DZY-1 can protect extrinsic gene from enzyme digestion during gene delivery and thereby promote gene delivery efficiency.

2.3 Observation of host and host-guest type gene delivery carriers interacting with DNA through SEM

We used SEM to observe the structures of complexes which were formed when host and host-guest type gene delivery carriers interacted with DNA. In λ-DNA/DZY-1 and λ-DNA/DZY-(AM) systems, the corresponding concentration ratio of DNA to carriers was 1∶300. The reaction time is 30 min. The SEM image of the structure of complexes is shown in Fig. 6.

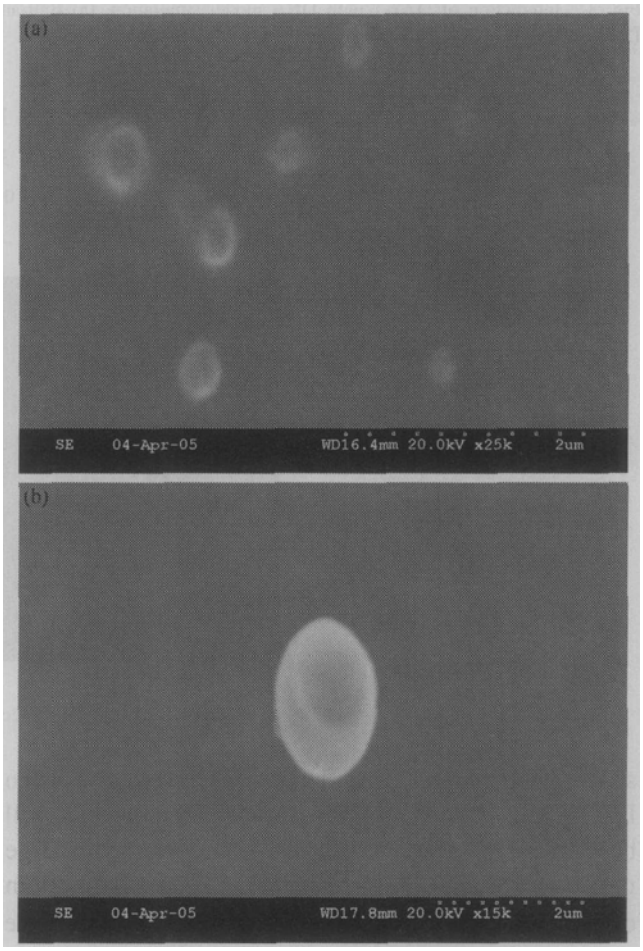

Fig. 6. SEM images of interactions between DZY-1 and λ-DNA. (a) λ-DNA /DZY-1 system; (b) λ-DNA/(DZY-1/AM) system.

From the SEM image we conclude that in λ-DNA/DZY-1 system, DZY-1 interacted with λ-DNA to form condensates with circular and toroid structures and their diameters were from 200 to 400 nm (Fig. 6(a)); how-

ever, in λ-DNA/ (DZY-1/AM) system, the diameters of circular and toroid structures, which were formed due to the interactions between λ-DNA and (DZY-1/AM), are over 600 nm (Fig. 6(b)).

We suspect that when gene delivery carriers interact with DNA, the shapes and structures of DNA condensates vary with the concentration of carriers and reacting time. This is important to investigating the mechanism of DNA package, gene delivery and gene therapy *in vivo*. Related structure research about DNA condensate induced by host and host-guest gene delivery carriers with SEM and AFM, gene delivery research and gene express research in cells are under performance in our lab.

3 Conclusion

A host type non-virus gene delivery carrier with probing molecule was synthesized. Interactions between DZY-1 and DNA were characterized with electrophoresis assay. Experiment result indicates that the probing molecules can induce extrinsic DNA condensation and the β-cyclodextrin structure in host molecules can carry guest molecules (drugs or active components) and combine DNA to form super-molecule complexes. Guest molecules enhanced the formation of complexes. Super-molecule complexes formed at certain concentration can inhibit enzyme digestion. This proves that the host type non-virus gene delivery carrier can on one hand induce DNA condensation; on the other hand protect condensed DNA from enzyme digestion, which will greatly promote gene delivery efficiency. The experiment provides a new method for designing and producing non-virus gene delivery carriers.

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References

1. Bonadio, J., Goldstein, S. A., Levy, R. J., Gene therapy for tissue repair and regeneration, Advanced Drug Delivery Review, 1998, 33(1): 53－69.
2. Winn, S. R., Hu, Y. H., Sfeir, C. *et al*., Gene therapy approaches for modulating bone regeneration, Advanced Drug Delivery Review, 2000, 42: 121－138.
3. Parker, A. L., Oupicky, D., Dash, P. R. *et al*., Methodologies for monitoring nanoparticle formation by self-assembly of DNA with poly (L-lysine), Anal. Biochem., 2002, 302(1): 75－80.
4. Vijayanathan, V., Thomas, T., Thomas, T. J., DNA nanoparticles and development of DNA delivery vehicles for gene delivery, Biochemistry, 2002, 41(48): 14085－14094.
5. Mckenzie, D. L., Kwok, K. Y., Rice, K. G., A potent new class of reductively activated peptide gene delivery agents, J. Biol. Chem., 2000, 275(14): 9970－9977.
6. Scherer, F., Anton, M., Schillimger, U. *et al.*, Magnetofection: enhancing and targeting gene delivery by magnetic force *in vitro* and *in vivo*, Gene Ther., 2002, 9(2): 102－109.
7. Brunner, S., Sauer, T., Carotta, S. *et al*., Cell cycle dependence of gene transfer by lipoplex, polyplex and recombinant adenovirus, Gene Therapy, 2000, 7(5): 401–407.
8. Sakurai, F., Inoue, R., Nishino, Y. *et al.,* Effect of DNA/Liposome mixing ratio on the physicochemical, characteristics, cellular uptake, and intracellular trafficking of plasmid DNA/cationic liposome complexes and subsequent gene expression, J. Controlled Release, 2000, 66(3): 255－269.
9. Wagner, E., Application of membrane-active peptides for nonviral gene delivery, Advanced Drug Delivery Review, 1999, 38(3): 279－289.
10. Huang, S. W., Zhuo, R. X., Recent progress in polymer-based gene delivery vectors, Chinese Science Bulletin, 2003, 48(13): 1304－1309.
11. Wang, Y. X., Shen, J. C., Progress in non-viral gene delivery systems fabricated via supramolecular assembly, Chinese Science Bulletin, 2005, 50(4): 289－294.
12. Lee, J. W., Selvapalam, S. N., Kim, H. J. *et al*., Cucurbituril homologues and derivatives: New opportunities in supramolecular chemistry, Acc. Chem. Res., 2003, 36: 621－630.
13. Engeldinger, E., Armspach, D., Matt, D., Capped cyclodextrins, Chem. Rev., 2003, 103(11): 4147－4173.

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