

# Synthesis and characterization of CD44 targeted nanoparticle

## PEI/pDNA/HA-mPEG for gene delivery

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**Abstract.** In order to prepare targeted nanocarrier to gene delivery, a novel nanoparticle was designed to condense pDNA. Hyaluronic acid was regarded as the targeted bio-materials because the HA-receptor-mediated CD44 receptors existed in many issues which were interacted with tumor membrane. The preparation procedure was determined by N/COOH and condensing pDNA capability was measured with agarose gel electrophoresis assay. The HA-mPEG polymer was characterized by FI-IR and PEI/pDNA/HA-mPEG nanoparticle was characterized by Nano Zetasizer. The obtained results was showed that the HA-mPEG was successfully synthesized and the ternary complexes presented relatively uniform particle distribution with 200-450 nm, the zeta potential was increased with the increasing content of PEI. The PEI/DNA/HA-mPEG nanoparticle showed the potential in gene delivery.

### Introduction

Gene delivery has been the most potential instrument to treat acquired or congenital diseases when the traditional methods was limited by their inability [1, 2, 3], the findings of new vectors have received an increasing enthusiasm by researches. Among the various cationic polymer, polyethlenimine (PEI) was candidated as one of the most effective gene delivery carriers in condensing negative charged DNA or siRNA, Branched PEI is widely used in delivery DNA for their high levels of gene expression [4, 5, 6]. PEI could interacted with DNA or polyanions through electrostatic interactions, the polyanions reduced the cytotoxicity of the complexes with its negative charge. As one of the polyanions, hyaluronic acid (HA) was investigated as bio-materials due to its biocompatibility, physicochemical properties, biological effects [7, 8]. The abundant HA receptors can be recognized by cancer cells and enhanced the HA-receptor-mediated endocytosis [9], especially in the liver. HA prevented non-specific binding in serum protein and enhanced its targeting. PEG derivatives HA-mPEG could reduce the ternary complexes surface charge and prolonged the circulation of blood [10].

In this work, the PEI/pDNA/HA-mPEG nanoparticle was synthesized through the electrostatic interactions between the positive charge of PEI and negative charge of DNA and HA-mPEG (Fig.1). The size of PEI/DNA/HA-mPEG nanoparticle at different N/COOH ratios was ranging from 244 to 416nm, the zeta potential was -22.9mV to 36.3 mV. The agarose gel electrophoresis assay of PEI/pDNA/HA-mPEG nanoparticle at various N/COOH ratios was condensing DNA with different condensing capability. The PEI/DNA/HA-mPEG nanoparticle might be useful gene carrier in gene delivery with CD44 targeted material.

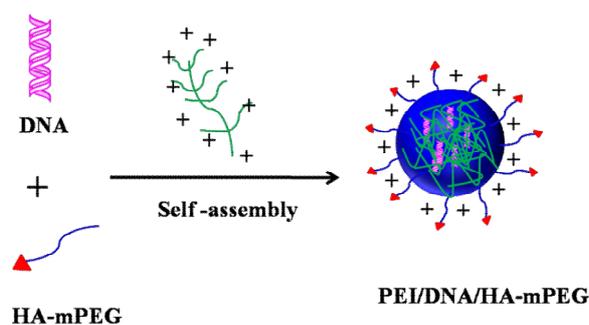


Fig.1 The synthesis procedure of PEI/DNA/HA-mPEG nanoparticle

## Experimental sections

**Chemicals.** PEI (branched form, average molecular weight (MW) of 25kDa) was purchased from Sigma Aldrich Chemical Co. (Milwaukee, WI, USA). Hyaluronic acid (HA) with a molecular weight (MW) of 7kDa was purchased from shanghai huilang industrial Co (shanghai, China). Monomethoxyl poly(ethylene glycol) (mPEG,  $M_n=2kDa$ ), ethylenediamine, NaOH, HCl, 4-Toluene sulfonyl-chloride (TsCl) was purchased from Aladdin Chemistry Co. China, N-hydroxysuccinimide (NHS) (95-99%) were purchased from Sinopharm Chemical Reagent, 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC·HCl) was purchased from Shanghai Medpep Co Ltd, China. All solvents were of analytical grade and used without further treatment.

**Synthesis of hyaluronic acid targeted copolymers (HA-mPEG).** The HA-mPEG copolymer was synthesized as shown in Fig.2. 4g (2.0mmol) of mPEG ( $M_w = 2000$ ) in 5mL of THF were added to a solution of 400 mg (10mmol) of NaOH in 20 mL of Milli-Q water. The resulting mixture was stirred for 1 h at 0°C. Then 0.5g (2.5mmol) of TsCl in 5 mL of THF was added dropwise to the reaction mixture during 1 h at 0°C. After the mixture was stirred for another 3 h at room temperature, the solution was poured into 10 mL of 1 M HCl in beakers and the organic solvent was evaporated in a vacuum. The residue was extracted with 25 mL of dichloromethane 3 times, and the organic phase was dried over  $MgSO_4$ , followed by filtration and the solvent was removed by rotary evaporation. The 2g transparent crude product was reacted with 0.619 mL ethylenediamine (9.28mmol, excess) in 50mL of chloroform for 8 h under reflux conditions. The organic solvent was removed by rotary evaporation to obtain the raw product. The product was precipitated with cold ether and dried under vacuum to give 1.8 g of mPEG-NH<sub>2</sub> which was stored at 4 °C.

HA (0.233g, 0.578mmol) and EDC (0.265g, 1.38mmol), NHS (0.159g, 1.38mmol) were dissolved in 5mL Milli-Q water and stirred for 1h, the active HA was obtained for the following reactions. The mPEG-NH<sub>2</sub> (1.156 g, 0.578mmol) was dissolved in the 10ml Milli-Q water, and the active HA added drop-wise to the mPEG-NH<sub>2</sub> solution, then stirred for further 24 h. The unreacted HA, mPEG-NH<sub>2</sub>, EDC and NHS were removed by dialysis against Milli-Q water. The product hyaluronic acid targeted copolymers was collected by lyophilization (Fig.2).

**Construction of pDNA.** Enhanced green fluorescent protein (GFP) encoding the plasmid (pEGFP-C1) and was presented from the team of Binlei Liu professor. The pDNA was amplified using an EndoFree Plasmid Maxi Kit (Omega, USA). The pDNA was dissolved in DNA wash buffer and stored at -20°C until use.

**Preparation of complexes.** The theoretical charge ratios of PEI/pDNA/HA-mPEG was calculated as the molar ratio of PEI nitrogen to HA carboxylate. To prepare the ternary complexes containing pDNA, an appropriate amount of stock PEI solution (0.5mg/mL, pH7.4) was mixed with HA-mPEG (1mg/ml, pH9.0) and pDNA mixture by drop-wise to prepare PEI/

pDNA/ HA-mPEG nanoparticle at different ratios while the concentration of pDNA was kept constant, the mixture was added drop-wise to PEI solution, the total volume for each sample was 100 $\mu$ L. The resultant mixtures were vortexed for 30s and incubated at 37 $^{\circ}$ C for 1h.

**Gel Retardation Assay.** To evaluate the condensing ability of the polymers with pDNA, electrophoresis was performed. A volume of 10  $\mu$ L of well-incubated complexes solution containing 0.2 $\mu$ g of pDNA were mixed with 2  $\mu$ L of 10 $\times$ loading buffer (Takara Biotechnology, Dalian, China), then the suspension were loaded onto 0.8% agarose gel containing 5 $\mu$ g/mL ethidium bromide and the electrophoresis was carried out at a voltage of 110 V in 1 $\times$  TAE running buffer for 30min. The pDNA retardation was analyzed on an image master VDS thermal imaging system (BioRad, CA) at a UV light wavelength of 254 nm.

**Characterization of PEI/pDNA /HA-mPEG nanoparticle.** The surface electrostatic properties (Zeta potential) and dynamic light scattering (DLS) measurements were examined using MALVERN Zetasizer ZS90. Fourier transform infrared (FT-IR) spectrum was recorded on Nicolet Nexus 470.

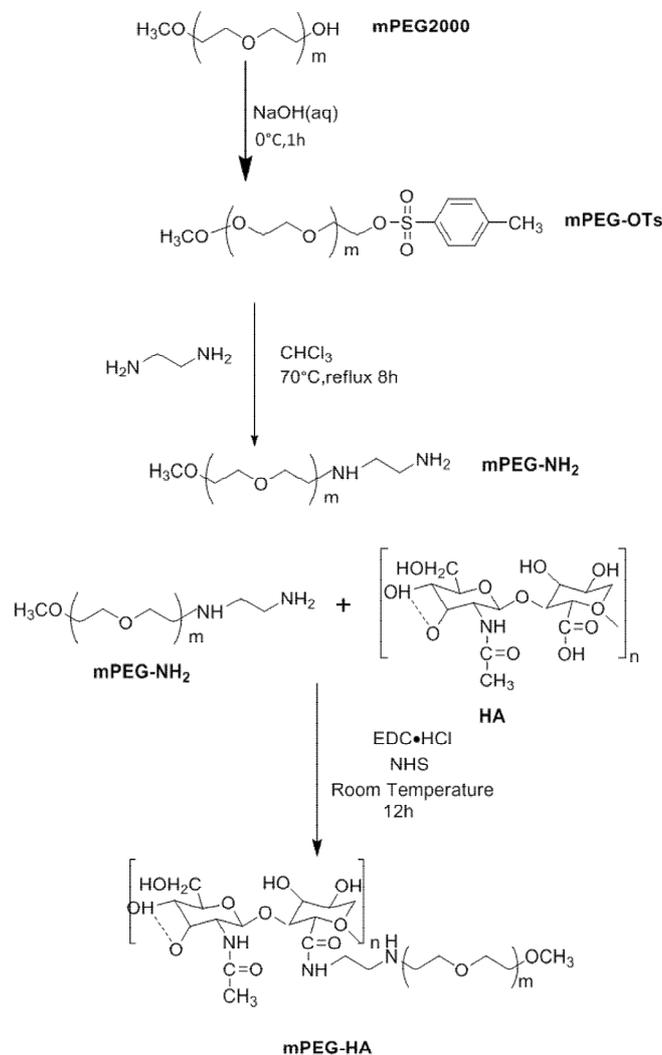


Fig.2 The synthesis procedure of HA-mPEG

## Results and discussions

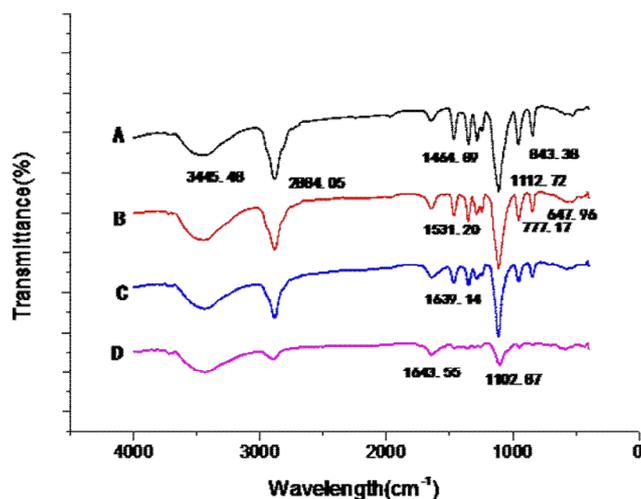


Fig.3 FT-IR spectra of mPEG2000 (A) , mPEG –OTs (B), mPEG –NH<sub>2</sub>(C), HA- mPEG (D).

The FT-IR spectras of mPEG2000 (A) , mPEG–OTs (B), mPEG –NH<sub>2</sub>(C), HA- mPEG (D) was shown in Fig.3, 2884.05cm<sup>-1</sup>, 1464.89cm<sup>-1</sup>, 843.38cm<sup>-1</sup> was assigned to stretching, bending and vibrational peaks of C-H groups. The O-H band at 3445.48cm<sup>-1</sup>and C-O stretching band at 1112.72cm<sup>-1</sup>was observed in mPEG2000.The new adsorption peak appeared at mPEG –OTs ,647.96cm<sup>-1</sup>,777.17cm<sup>-1</sup>,1531.20cm<sup>-1</sup> was appeared at S-O stretching, Cyclobenzene and S=O stretching adsorption peaks. The N-H stretching adsorption peaks was observed at 1639.14cm<sup>-1</sup>and the C=O adsorption peaks 1643.55cm<sup>-1</sup> was observed in HA- mPEG, therefore the HA- mPEG was successfully synthesized with the methods mentioned above.

Table 1 Particle Size and Zeta potential of PEI/pDNA /HA-mPEG nanoparticle

N/COOH	Size[nm]	PDI	Zeta potential[mV]
0.5:1	416.2	0.362	-22.9
2.5:1	244.0	0.294	28.4
5:1	299.0	0.417	30.2
10:1	267.4	0.381	36.3

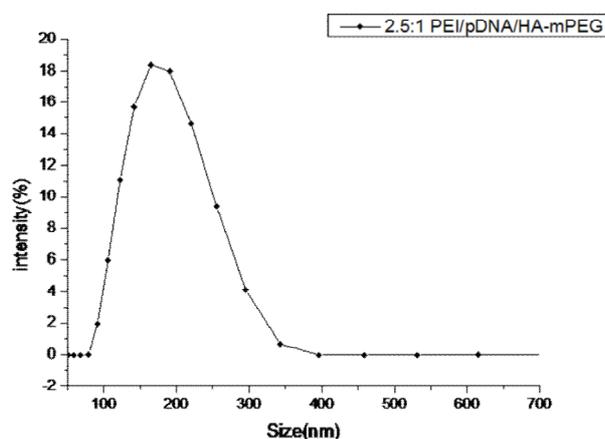


Fig.4 Hydrodynamic diameter of PEI/pDNA /HA-mPEG nanoparticle of N/COOH at a charge ratio of 2.5

The different N/COOH of PEI/ pDNA /HA-mPEG nanoparticle was synthesized with the methods mentioned above (Fig.1), the hydrodynamic diameter of ternary complexes was determined by DLS (Table 1) and the increasing of N/COOH which was induced the increasing of zeta potential, the foundation for electrostatic interactions with pDNA and HA-mPEG. As shown in Fig.4, the size of N/COOH at a charge ratio of 2.5 was 244.0 nm.

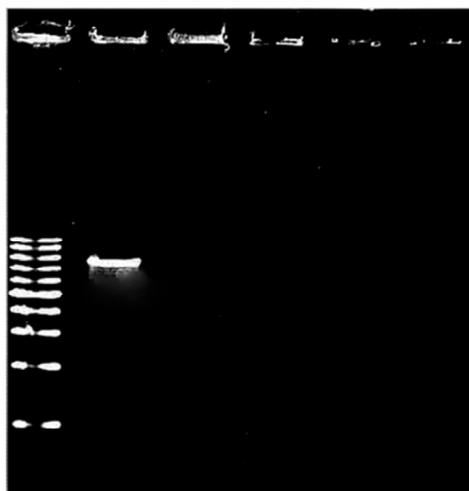


Fig .5 Agarose gel electrophoresis assay of PEI/pDNA /HA-mPEG nanoparticle at various N/COOH ratios

The DNA condensation capacity of PEI/pDNA /HA-mPEG nanoparticle at various N/COOH ratios was measured by agarose gel electrophoresis assay, as show in Fig.5, pDNA release was not observed.

## Conclusions

In this work, the PEI/pDNA/HA-mPEG nanoparticle was successfully synthesized with the methods mentioned above, the different N/COOH of ternary complexes was controlled the nanoparticle size and zeta potential .the three steps of synthesis HA-mPEG polymer was determined by FI-IR , the characteristic absorption peak was observed . The DNA condensation capacity was showed polyethylenimine which was the cationic polymers condensing DNA with electrostatic interactions with negative group.

## Acknowledgements

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