Supramolecular nanoparticles based on β-CD modified hyaluronic acid for DNA encapsulation and controlled release†

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The construction of targeted drug/gene nanocarrier systems through non-covalent interactions has become an emerging strategy for researchers in the chemical and biological/medicinal materials fields.1–4 The convenient chemical synthesis of building blocks, controllable assembly, large capacity for loading of drugs/genes, and controllable release of cargo are possible advantages of nano-supramolecular delivery systems.5–11 Among a series of supramolecular interactions, the binding behavior between adamantane and β-cyclodextrin has often been selected due to the large association constant and high reliability of the interactions.12–15 Recently, several targeted drug/gene delivery systems based on adamantane–β-cyclodextrin interactions were reported by our group and other scientific researchers.16–19 Zhang et al.20 have reported a targeted siRNA delivery nanoparticle constructed by supramolecular interactions, and the siRNA binding behavior with an adamantane–bis(diamine) conjugate was enhanced by a cucurbit[6]uril complexation-induced pH shift. Moreover, Zhao’s group21 have reported a novel mesoporous silica nanoparticle functionalized with β-cyclodextrin by cleavable disulfide bonds, which could hold anti-cancer drugs inside the nanoparticle. PEG-folate modified adamantane was also introduced onto the surface of the silica nanoparticle to improve the biocompatibility and targeting capabilities through supramolecular interactions. Finally, controllable drug release was realized by cleavage of disulfide bonds by glutathione in vitro.

On the other hand, one problem in targeted gene delivery is the timely release of the loaded pDNA/siRNA, which might be the determinant for the further promotion of gene transfection efficiency.22–24 A number of literature studies pay attention to the effective condensation and loading of nucleic acids, however, the stimulus-response release of the nucleic acids when they arrive at the targeting zone should be also considered.25,26 To solve this problem, Jiang’s27–32 group reported a molecular structure with a connected carboxylic ester group and a positively charged quaternary ammonium group together, and this structure could bind negatively charged biomolecules, such as nucleic acids, proteins, phospholipids, etc. When the ester group was hydrolyzed to a negative charged carboxyl, a positively charged group and a negatively charged group simultaneously appeared on one chain, which was deemed to be a “zwitterionic” molecule and was considered as an ultralow-fouling material, and the packaged biomolecules were effectively released.

In this work, we synthesized a doubly positively charged guest, ADA2+, bearing one adamantyl group on one side and two quaternary ammonium chains on the other side, and then fabricated β-CD modified hyaluronic acid (HACD) which was reported by our group previously. When ADA2+ and HACD were mixed together, the nanoparticle named ADA2+@HACD, which had a negatively charged HA shell and a positively charged ammonium chain core, was constructed through the supramolecular interactions between the adamantane group in ADA2+ and the β-CD group in HACD (Scheme 1). The positive core of the ADA2+@HACD nanoparticles could bind negatively charged plasmid DNA (pDNA), which then could be released when the ester group on the quaternary ammonium chain of ADA2+ was hydrolyzed to form a zwitterionic group, and controllable pDNA binding and release by the ADA2+@HACD nanoparticles could be realized.

HACD was synthesized according to our previous work.33,34 The molecular weight of HA that we employed in this work was 290 kDa, and the degree of substitution on HACD was calculated as one β-CD unit grafted on every seven HA repeating sugar

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units on average. This was determined according to the $^1$H NMR spectra of HACD, and meets the requirements that at least six successive repeating units of HA are needed as one targeting section for achieving multiple HA–CD44 interactions in tumor-targeting bioconjugates for drug/gene delivery. On the other hand, the guest molecule ADA2+ bearing one adamantyl group and two quaternary ammonium chains was synthesized through a five-step synthetic route (Fig. S1, ESI†) with a total yield of 71%. The characterization of the intermediate products (compound 1–4) and ADA2+ is presented in Fig. S2–S16 (ESI†). As shown in Scheme 1, the adamantyl group in ADA2+ could be included in the cavity of β-CD of HACD to form ADA2+@HACD nanoparticles, and the double quaternary ammonium chain could electrostatically interact with the anionic phosphate backbones of pDNA. The bound pDNA could be released via the hydrolysis of the ester group on the quaternary ammonium chain in ADA2+ by forming a zwitterionic structure.

We employed β-CD as a reference host and then used a $^1$H NMR titration method to investigate the supramolecular interactions between the adamantane part of ADA2+ and the β-CD moiety in HACD. As shown in Fig. 1a, with the increase of the concentration of β-CD from 0–9 mM, the proton signals of ADA2+ (1 mM) shifted downfield from $\delta = 2.01$ ppm to $\delta = 2.22$ ppm, accompanied by shape changes, which indicated the inclusion of the adamantane part of ADA2+ into the cavity of β-CD. Furthermore, by analyzing the nonlinear least-squares fit of the titration data (Fig. 1b), the binding constant ($K_a$) between ADA2+ and β-CD was calculated as $(6.1 \pm 0.8) \times 10^3$ M$^{-1}$. Moreover, the stoichiometry between ADA2+ and β-CD was determined as 1:1 according to the Job plot in which the maximum appeared at a molar fraction of 0.5 (Fig. S17, ESI†).

Taking advantage of the strong supramolecular interactions between adamantane and β-CD, the supramolecular nanoparticle ADA2+@HACD was obtained by simply mixing aqueous solutions of ADA2+ and HACD together. The morphology, size, and surface charge of the ADA2+@HACD nanoparticles were characterized by atomic force microscopy (AFM), high-resolution transmission electron microscopy (HR-TEM), dynamic light scattering (DLS), and ζ potential experiments. As shown in Fig. S18a (ESI†), the guest molecule ADA2+ could form spherical self-assemblies whose diameter was measured as ca. 600 nm, and the height of the loose and collapsed assembly was obtained as 2.7 nm. However, in Fig. 2a, after complexation with HACD, spherical shaped ADA2+@HACD nanoparticles were observed with a diameter of around 120 nm, and the height of the collapsed nanoparticle was measured as 6.5 nm, which was roughly equal to the sum of the values of two HA backbones (ca. 1.5 nm), two β-CD units (ca. 1.6 nm), and two flexible double quaternary ammonium chains with bromide counter anions (3.5 nm). The HR-TEM image (Fig. 2b) also showed the 100–130 nm homogeneous
spherical nanoparticles with homogeneous dispersity. Moreover, as shown in Fig. 2c, the hydrodynamic diameter of the ADA2+@HACD nanoparticles was measured by DLS experiments as ca. 286 nm, with a narrow distribution, which was much less than that of the self-assemblies of ADA2+ of ca. 616 nm (Fig. S18b, ESI†). Finally, the surface charges of the ADA2+ self-assemblies and the ADA2+@HACD nanoparticles were measured using ζ potential experiments. As shown in Fig. S18c (ESI†), the surface charge of the ADA2+ self-assemblies was measured as ca. +23 mV, implying that the quaternary ammonium chains, with positive charges, pointed toward the outside of the ADA2+ self-assemblies. After addition of HACD, as shown in Fig. 2d, the resulting ADA2+@HACD nanoparticles showed a typical negative charge on the surface of ca. −17 mV, indicating the coverage of negatively charged HA on the surface of the ADA2+@HACD nanoparticles. Subsequently, according to the results we discussed above, we could conclude that the self-assembly of ADA2+ which had a positively charged hydrophilic quaternary ammonium chain surface, and a hydrophobic adamantane core, could be formed in aqueous solution in an incompact way due to the amphiphilic nature of ADA2+. After inclusion of the adamantane part of ADA2+ into the cavity of β-CD in HACD, the ADA2+@HACD nanoparticles could be constituted, which had a negatively charged HA shell, and a positively charged quaternary ammonium chain core. The resulting ADA2+@HACD nanoparticles could not only recognize the HA receptor positive cancer cells, but also reduced the cytotoxicity of ADA2+ because of the cytomembrane protection from the cytotoxic positively charged HA shell.

Furthermore, the formation of ADA2+@HACD nanoparticles could be also recognized by the naked eye. As shown in Fig. S19 (ESI†), ADA2+, HACD, and ADA2+@HACD nanoparticles existed as steady and transparent aqueous solutions. Among them, ADA2+ exhibited the strongest Tyndall effect due to the largest size (ca. 600 nm) of the self-assembly in aqueous solution. ADA2+@HACD nanoparticles showed much more of an obvious Tyndall effect than HACD, which indicated the formation of nanoscale particles with modest sizes (ca. 130 nm).

Due to the quaternary ammonium chains of ADA2+ with positive charge in aqueous solution, ADA2+ was considered to bind DNA through electrostatic interactions. Subsequently, the pDNA binding behaviors of ADA2+ and ADA2+@HACD nanoparticles were examined by electrophoresis experiments by analyzing the electrophoretic mobility at different N/P ratios (the charge ratio of the cationic quaternary ammonium units in ADA2+ to the anionic phosphate units in pDNA). As shown in Fig. 3a, the pDNA was completely retarded by ADA2+ at N/P ≥ 30. After adding HACD, the negatively charged HA chain did not impede the pDNA binding behavior of ADA2+, and the formed ADA2+@HACD nanoparticles exhibited similar pDNA binding capabilities as ADA2+, thus the minimum N/P ratio for complete pDNA retardation was also observed as 30 (Fig. 3b). This phenomenon might be attributed to the encapsulation of pDNA into the ADA2+@HACD nanoparticles, which had a negatively charged HA shell, and a positively charged quaternary ammonium chains core, and the HA chains had no interfering electrostatic interaction with ADA2+.

On the other hand, the controllable pDNA release by hydrolysis of ester groups in ADA2+ was performed by electrophoresis experiments of pDNA–ADA2+ and pDNA–ADA2+@HACD adducts before and after hydrolysis with aqueous NaOH solution. As shown in Fig. 4, at N/P = 30, ADA2+ and ADA2+@HACD nanoparticles showed satisfactory pDNA retardation effects (line 1 and line 3, respectively), due to the electrostatic interaction between the positively charged quaternary ammonium in ADA2+ and the negatively charged phosphate of the pDNA. After hydrolysis with NaOH aqueous solution, the neutral ester group in ADA2+ changed to a negatively charged carboxyl group, and a zwitterionic ADA2+ appeared which had a positive charge and a negative charge on one chain. This zwitterionic species was highly resistant
to bio-molecular absorption, and pDNA release from ADA2+ and ADA2+@HACD were observed (line 2 and line 4 in Fig. 4). The process of pDNA binding and release is exhibited in Scheme 2. 

In conclusion, a targeted pDNA encapsulation system of ADA2+@HACD nanoparticles was successfully constructed by ADA2+ and HACD through supramolecular interactions. The nanoparticles were well-characterized, and had negatively charged HA shells and positively charged quaternary ammonium chain cores. The nanoparticles could bind pDNA effectively. Moreover, after hydrolysis of the ester group in ADA2+, the quaternary ammonium chain with a positive charge was converted to a zwitterionic group, and the encapsulated pDNA could be released. The controllable binding and release of pDNA by this approach might give important insights into methods of gene transfection of target cells. Finally, the construction of ADA2+@HACD nanoparticles provides an attractive strategy and platform for targeted delivery, and controllable packaging and release of pharmaceutical nucleic acids. Continual optimization of the quaternary ammonium chains in ADA2+ is the goal of endeavor for our group. 

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Conflicts of interest

The authors declare no competing financial interest.

Notes and references

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