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Letter

# Enhanced structural stability of adenovirus nanocapsule

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**Abstract**

Application of viral vector in gene therapy and vaccination is still limited by their structural stability, which significantly increased avoidable cost in storage and transportation. Herein a non-covalent conjugated low-pH degradable nanocapsule has been adopted to stabilize viral vectors. By utilizing a luciferase expressing adenovirus, AdCMVLuc, we succeeded in a raise of over 11 folds in AdCMVLuc's structural stability after 12 days storage at 4 °C.


*Keywords:* Non-covalent nanocapsule; Adenovirus; Structural stability

## 1. Introduction

Adenovirus is one of the most commonly used DNA viral vectors and widely accepted to be a safe and effective gene carrier in vaccination and gene therapy trials [1–7]. However, most of the currently available live viral vaccines are lyophilized products, and none of them provides sufficient structural stability to obviate the manufacturers' requirements for storage at 4–8 °C or even below −20 °C. At current state of the art, cold storage is major preference for viral vectors, however even in the developed world, the cold chain accounts for up to 14% of the vaccination costs [8,9]. More importantly, recent studies showed that adenovirus with alternated genome, usually smaller than wide-type's, presented dramatically lower structural stability than original adenovirus [10,11]. Therefore developing structural stabilization technologies of adenovirus based vaccine or gene therapy vectors would significantly reduce the cost of delivery as well as increase the overall efficacy especially for the developing world [12].

Here, we present a facile approach to synthesize highly stabilized adenovirus nanocapsule through non-covalent interaction and *in situ* polymerization. The non-covalent electric interaction between positively charged monomers and negatively charged virion is utilized as anchors of polymer shell and acid degradable crosslinkers are added into co-polymerization. Therefore protective polymer shell will dissemble at low-pH in lysosome and adenovirus could be released. The proposed nanocapsule is a general solution to various viral vectors, as no specific binding site or modification on viral vector is required.

As illustrated in Fig. 1, lab-made positively charged monomers, NTris (please refer to materials and method), and low-pH degradable crosslinkers, glycerol dimethacrylate (GDMA), are *in situ* polymerized around adenovirus in an aqueous solution. The opposite charges on NTris and virion facilitate individual wrapping of each adenovirus. Such a crosslinked shell can protect the encapsulated adenovirus from structural denaturation and is readily degradable in acidic environment, such as pH 5.4. We believe that this unique, inducible design will provide adenovirus with outstanding stability during economical 4 °C storage and

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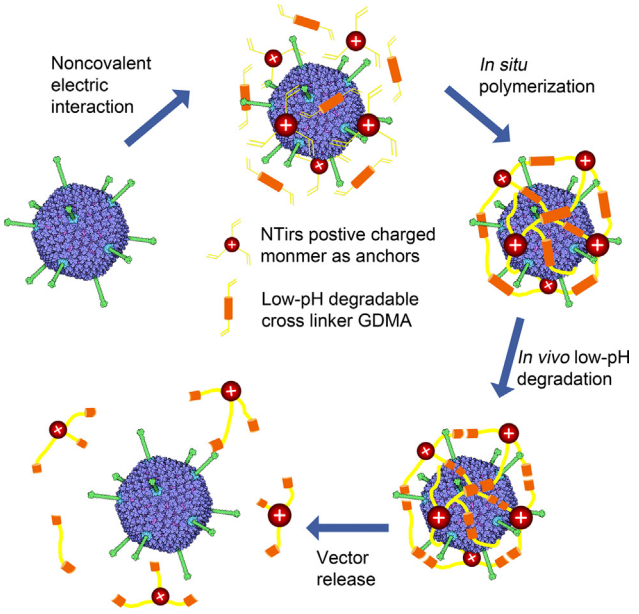


Fig. 1. *In situ* encapsulation of individual adenovirus and *in vivo* low-pH triggered viral release.

transportation, while enabling its release from the nanocapsule upon entering the acidic endosomal/lysosomal compartments. A luciferase expressing adenoviral vector, AdCMVLuc, is employed to demonstrate gene transfer efficiency of as-proposed nanocapsule *via* luciferase assay.

## 2. Materials and methods

### 2.1. Materials

All chemicals were purchased from Sigma-Aldrich unless otherwise noted, and were used as received. Luciferase assay kit was purchased from Promega, cat# E1501.

### 2.2. Synthesis of positively charged monomers

In a typical synthesis, 0.492 mmol of methacrylic anhydride and 0.615 mmol of triethylamine were dissolved in 1 mL of acetonitrile in the ice/water bath with vigorous stirring, marked as solution A. Then 20 mg tris(2-aminoethyl)amine was dissolved in another 1 mL of acetonitrile, dripped into solution A and reacted for 8 h. The solution was dried under vacuum and dark condition. After that, the compound was dissolved in 500 μL methanol and isolated by the silica gel chromatography, which mobile phase was chloroform and methanol with volume ratio 12:1. Final product was named as NTris for convenience. Purified NTris was dried under vacuum and dark condition then stored under −80 °C.

### 2.3. Synthesis of adenovirus nanocapsules

AdCMVLuc has been employed as the model delivery vector, also been referred as NativeAdv and the native adenovirus in later description. Typically, 10 μL of AdCMVLuc solution (0.5 mg/mL in 10 mM tris buffer with 1 mM $Mg^{2+}$, pH 8), 1 μL of NTris DMSO solution (4%), 1 μL of glycerol dimethacrylate (GDMA) DMSO solution (4%), 6.4 μL of ammonium persulphate in aqueous solution (dilute 100 times from fresh prepared 5 wt% aqueous solution), 6.4 μL of N,N,N′,N′-tetramethylethylenediamine aqueous solution (dilute 100 times from fresh prepared 1% aqueous solution) and 75.2 μL of tris buffer (10 mM tris buffer with 1 mM $Mg^{2+}$, pH 8) were mixed and reacted in ice bath for 2 h. Then adenovirus nanocapsules were dialyzed to the same tris buffer with $Mg^{2+}$.

### 2.4. Transmission electron microscope (TEM) imaging

TEM images of nanocapsules were obtained with a Philips EM120 Transmission Electron Microscope. Before the observation, nanocapsules were negatively stained using 1% pH 7.0 phosphotungstic acid (PTA) solution.

### 2.5. Zeta potential

Zeta potential and particle size distribution were measured with a Malvern particle sizer Nano-ZS. Size of adenovirus nanocapsule was characterized in 10 mM pH 8 tris buffer with 1 mM $Mg^{2+}$ and Zeta of adenovirus nanocapsule was collected in 2.5 mM pH 8 tris buffer with 0.25 mM $Mg^{2+}$.

### 2.6. Infectivity and structural stability of adenovirus nanocapsule in HeLa cells

- Day 1, adenovirus nanogels were synthesized and dialyzed with 10 mM tris buffer with 1 mM $Mg^{2+}$, pH 8 for overnight. 48 well plates were seeded with HeLa cells ($1 \times 10^4$ cell per well).
- Day 2, dialyzed adenovirus nanogel and native adenovirus were aliquot to 20 μL per 1.5 mL centrifuge tubes and stored at 4 °C. Firstly certain amount of native adenovirus and adenovirus nanocapsules were added into 48 well plate seeded on Day 1 to achieve MOI = 10, immediately after diluted to 5 times in volume with 1 M pH 5 acetate buffer. All the samples were tested in triplicates. Finally infected cell plate was incubated for 3 days and luciferase assays were carried out to determine infectivity.
- Day 5, luciferase assays were carried out to determine the infectivity of freshly prepared adenovirus nanogel.
- Day 11, 48 well plates were seeded with HeLa cells ($1 \times 10^4$ cell per well).
- Day 12, the same process of Day 2 was repeated.
- Day 15, luciferase assays were carried out for the infection on Day 12, to determine the stability of as-prepared adenovirus nanogel.

### 2.7. Luciferase assay

The standard protocol for Firefly Luciferase Assay kit (Promega, cat# E1501) was followed [13]. Briefly, infected cells were lysed and collected into 1.5 mL centrifuge tubes and centrifuged for