Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Series of new supramolecular polycations for effective gene transfection

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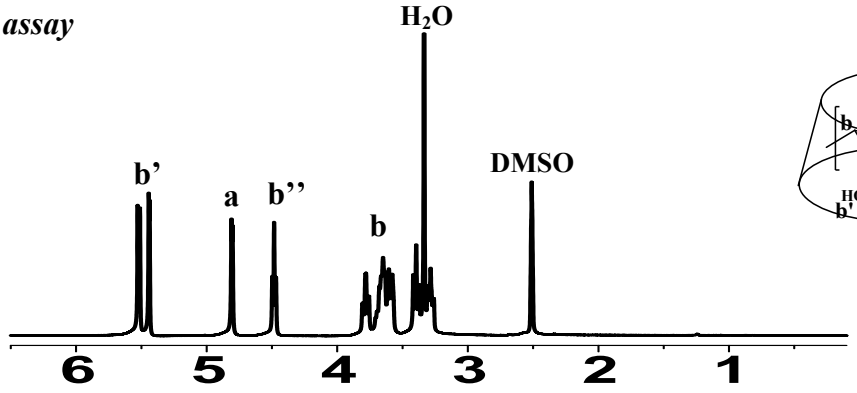
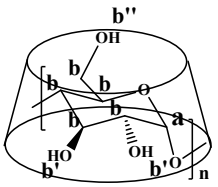
^{1}H NMR assay

(a) α-CD

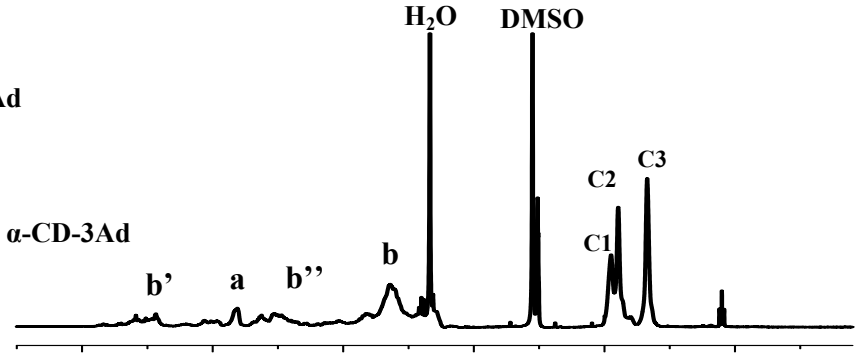


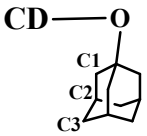


n=6 α-CD
n=7 β-CD

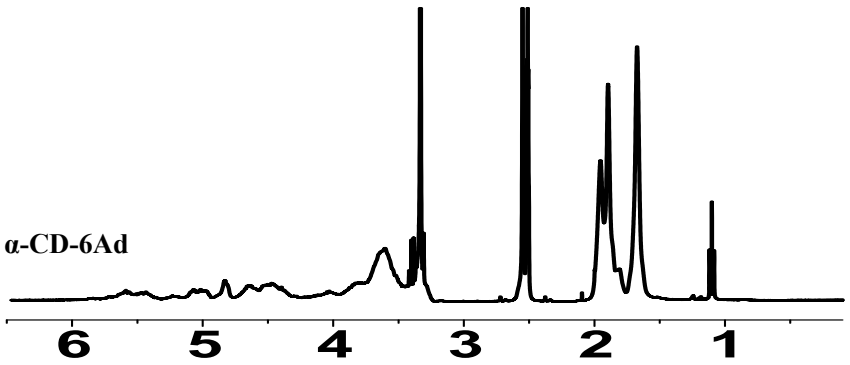
(b) α-CD-xAd

α-CD-3Ad

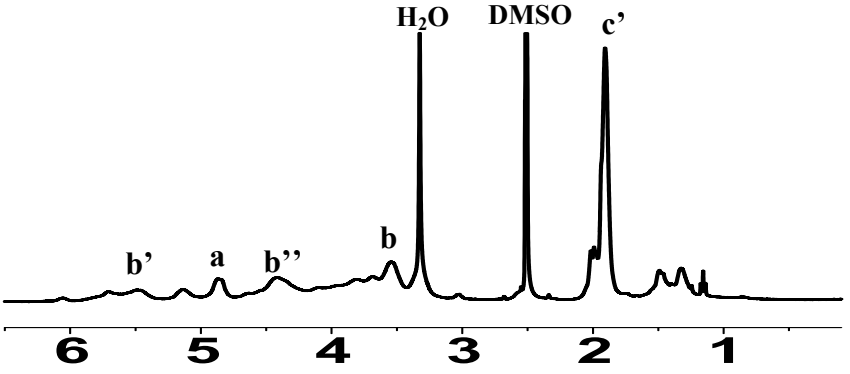


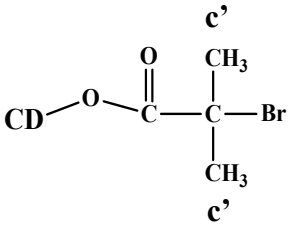


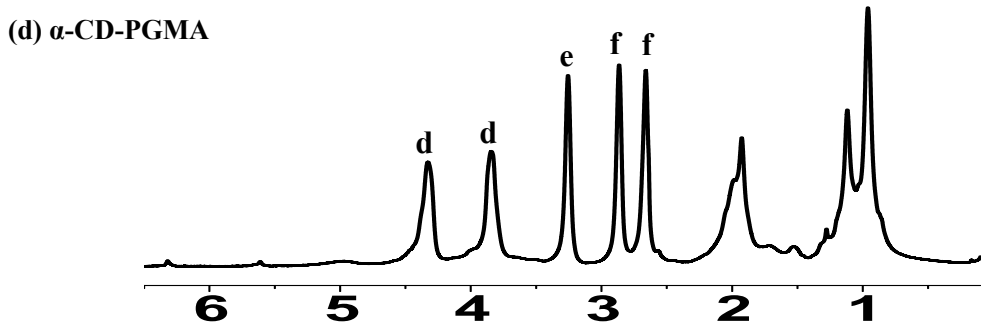
α-CD-6Ad

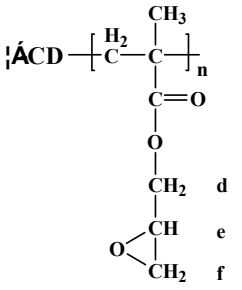


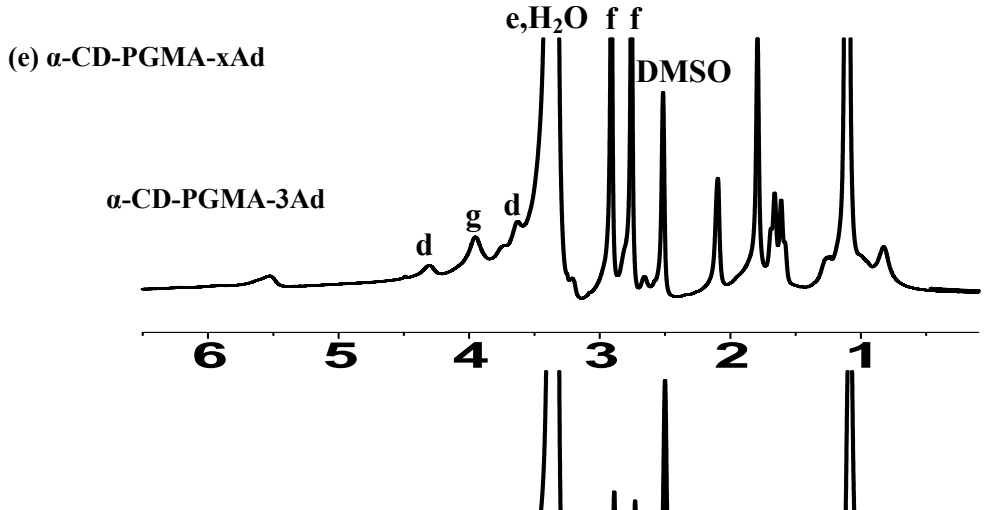
(c) α-CD-Br

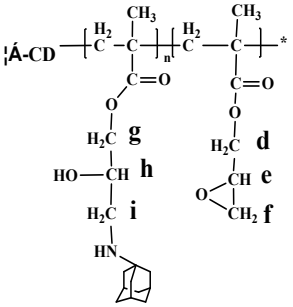


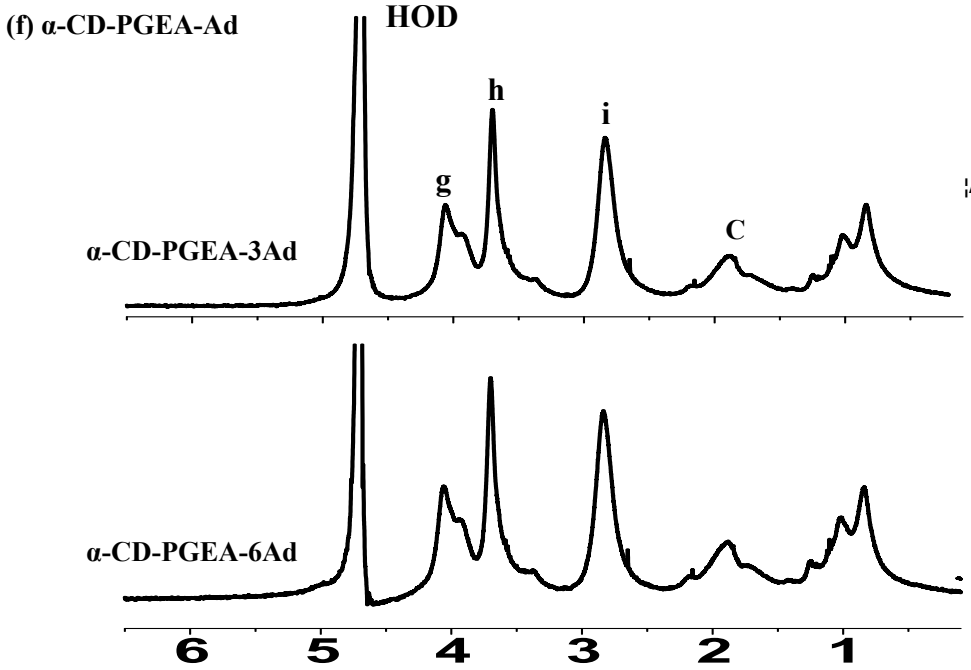


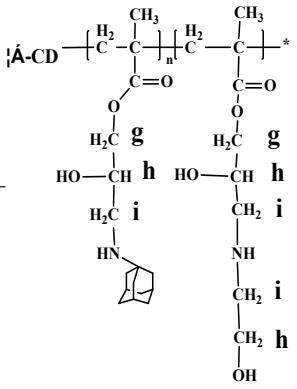












C: CH and CH_2 on Ad

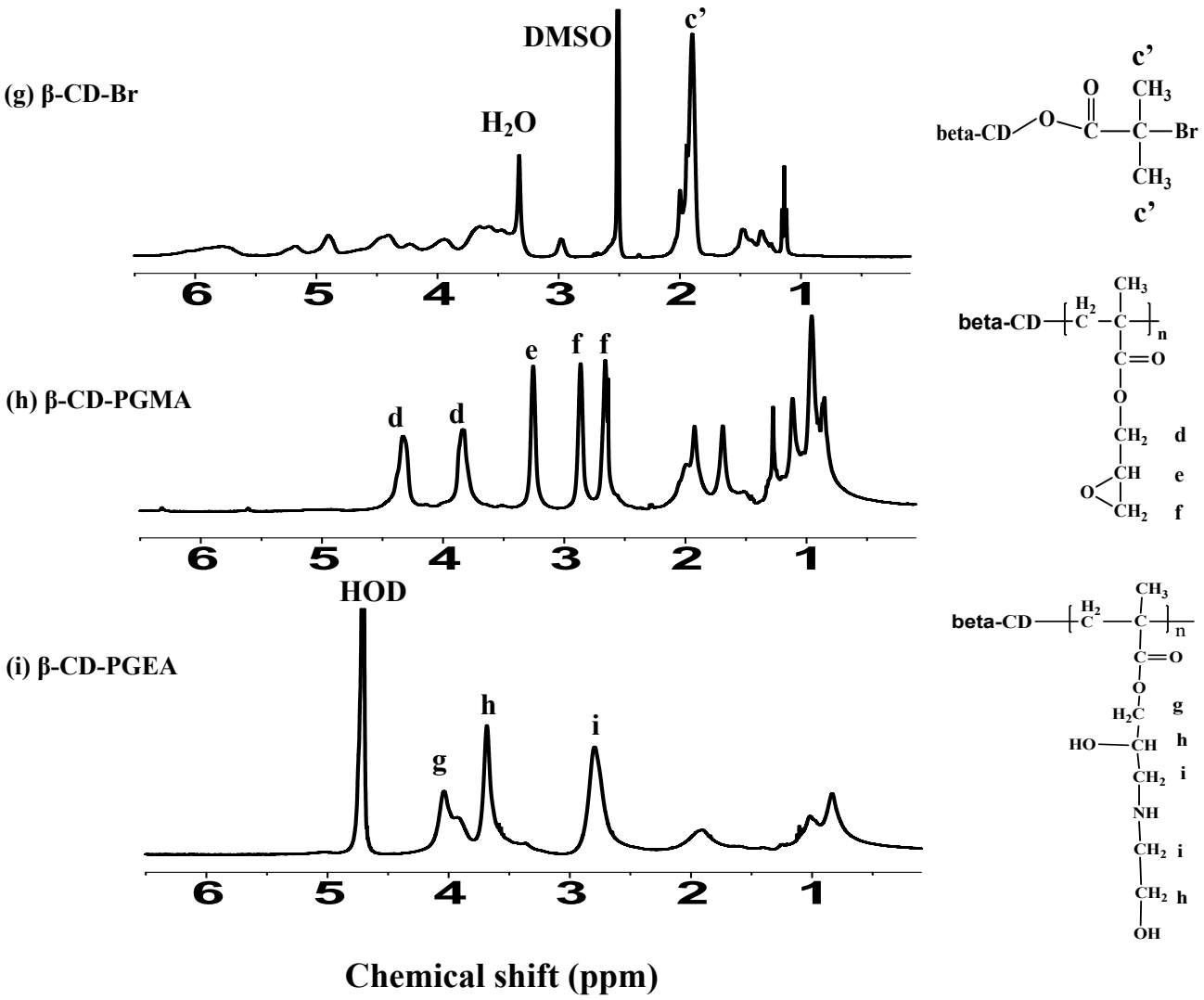


Fig.S1. 400 MHz ^{1}H NMR spectra of (a) α-CD in DMSO-d_6, (b) α-CD-Ad in DMSO-d_6, (c) α-CD-Br in DMSO-d_6, (d) α-CD-PGMA in $CDCl_3$, (e) α-CD-PGMA-Ad in DMSO-d_6, (f) α-CD-PGEA-Ad in D_2O, (g) β-CD-Br in DMSO-d_6, (h) β-CD-PGMA in $CDCl_3$ and (i) β-CD-PGEA in D_2O.

For α-CD (Fig. S1(a)), the signals located at broad chemical shifts in the region of 3.4−4.0 ppm are mainly associated with the inner methylidyne and methylene protons between the oxygen and carbon moieties (***b***, O-CH-C and O-CH_2-C) on the glucose units of α-CD. The peak located at a chemical shift of δ =4.88 ppm is attributable to the inner methylidyne protons between the oxygen moieties (a, O-CHO). The signals located at the chemical shifts in the region of 4.2-4.4 ppm are mainly attributable to the hydroxyl protons adjacent to the methylene moieties (***b''***, CH_2-OH). The peak at δ=5.75 ppm corresponds to the hydroxyl protons adjacent to the methylidyne moieties (***b'***, CH-OH) of glucose units, except the existence of H_2O peak (δ =3.33 ppm) in DMSO-d_6 solvent. For α-CD-Ad (Fig. S1(b)), the three chemical shifts in the region of 1.6-2.0 ppm were mainly associated with the inner methylidyne and methylene

protons (C: C1, CH-C methylidyne, δ =1.95 ppm; C2, CH-CH2-CH methylene, δ =1.89 ppm; C3, C(NH2)-CH2-CH2 methylene, δ =1.67 ppm;) on Ad. Based on the ratio of peak a and peak C1, C2 and C3, the content of Ad was calculated. The degree of substitution of the hydroxyl groups on the outside surface of α-CD is determined to be about 3.3 for α-CD-3Ad and 6.2 for α-CD-6Ad.

For α-CD-Br or β-CD-Br (Fig. S1(c) or (g)), the peak located at a chemical shift of δ=1.90 ppm is associated with the methyl protons (c', C(Br)-CH_3) of the 2-bromoisobutyryl groups. From the area ratio of peak c' and peak a, the degree of substitution of the hydroxyl groups on the outside surface of CD is determined to be about 3.0. The NMR results thus indicate that a CD-Br core with about three alkyl halide initiation sites has been successfully prepared.

For α-CD-PGMA or β-CD-PGMA (Fig. S1(d) or (h)), the signals at δ = 3.70 and 4.33 correspond to the methylene protons adjacent to the oxygen moieties of the ester linkages (d, CH2−O−C=O) of PGMA arms. The peaks at δ = 3.23 ppm (e) and δ = 2.67 and 2.82 ppm (f) could be assigned to the protons of the epoxide ring. The ratio of peak areas of peak e and peak f is about 1:2, indicating that the epoxy groups in the PGMA remained intact throughout ATRP, which was consistent with the earlier reports.[22,23] The signals associated with the CD core became less obvious, due to the minor contribution of CD to the overall star polymer structure. For β-CD-PGEA (Fig. S1(i)), peaks e and f associated with the epoxide rings of PGMA disappeared completely after the ring-opening reactions of PGMA with EA, and peaks a at 3.70 and 4.33 ppm shifted and combined to form a single peak at 4.0 ppm (g). The new peak at 3.70 ppm(h) is associated with the CH−OH methylidyne and O−CH_2 methylene protons. The strong peak at 2.84 ppm is mainly attributable to the methylene protons (i, NH−CH_2).

Two adamantine-modified α-CD-PGMAs (α-CD-PGMA-xAd) with different epoxy:Ad ratios (42:3 for α-CD-PGMA-3Ad; 42:6 for α-CD-PGMA-6Ad) were obtained via ring-opening reactions with amantadine (Ad-NH_2).The peak d at 3.70 and 4.33 ppm partly shifted and combined to form a single peak at 4.0 ppm (g). The peaks at δ = 3.23 ppm (e) and δ = 2.67 and 2.82 ppm (f) assigned to the protons of the epoxide ring were still retained. The Ad/α-CD ratios of α-CD-PGMA-3Ad and α-CD-PGMA-6Ad were 3:1 and 6:1, respectively.

For β-CD-PGEA (Fig. S1(i)) and α-CD-PGEA-Ad (Fig. S1(f)), peaks e and f associated with the epoxide rings of PGMA disappeared completely after the ring-opening reactions of PGMA with EA, and peak d at 3.70 and 4.33 ppm shifted and combined to form a single peak at 4.0 ppm (g). The new peak at 3.70 ppm (h) is associated with the CH−OH methylidyne and O−CH_2 methylene protons. The strong peak at 2.84 ppm is mainly attributable to the methylene protons (i, NH−CH_2). The above results indicated that the oxirane rings of PGMA were opened by EA under the present reaction conditions. For α-CD-PGEA-Ad (Fig. S1(f)), the obvious chemical shifts in the 1.6−2.1 ppm region are mainly associated with the inner methylidyne and methylene protons on the Ad units (C, CH and CH_2). Ad/α-CD ratios of α-CD-PGEA-3Ad and α-CD-PGEA-6Ad were calculated to be 3:1 and 6:1, respectively.

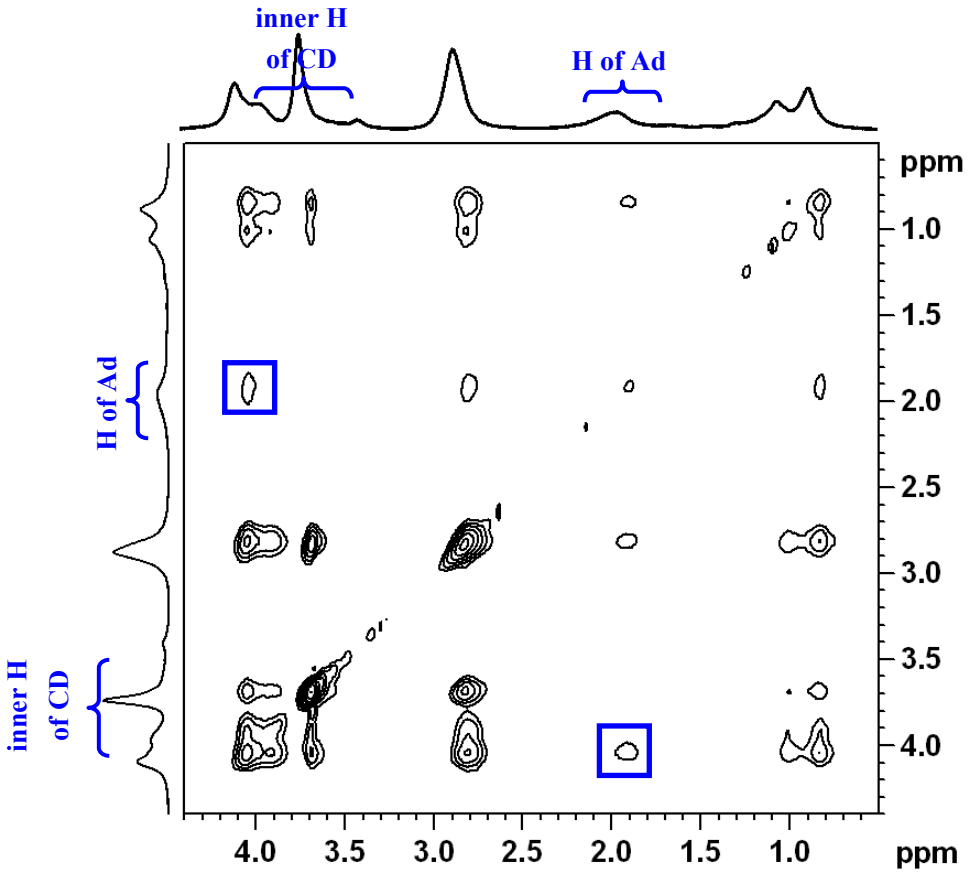


Fig.S2. 2D NOESY ^{1}H NMR spectrum of α-CD-6Ad/β-CD-PGEA.

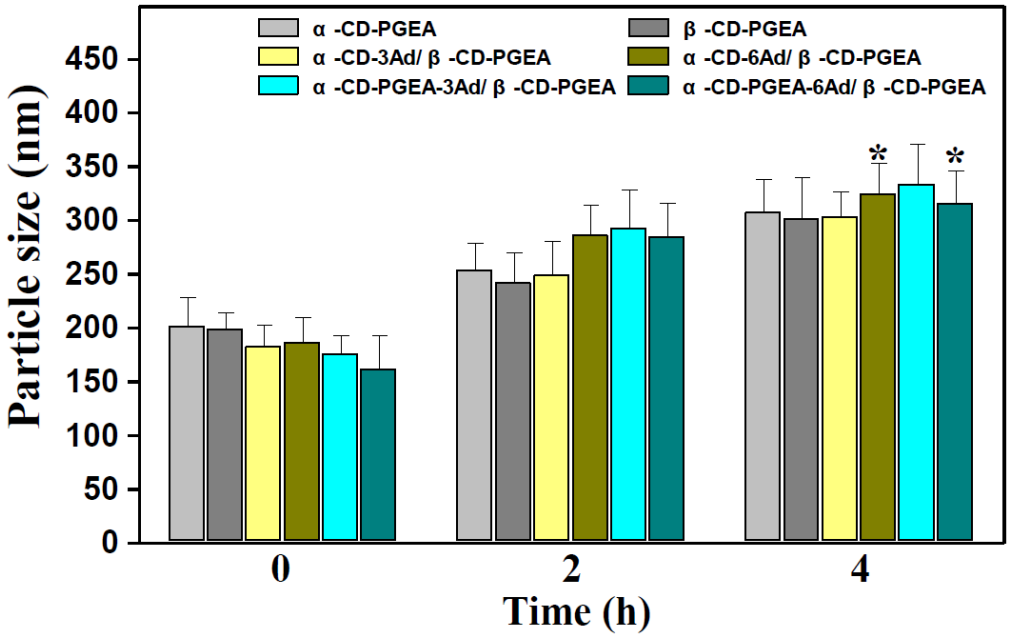


Fig. S3. Particle sizes of the polycation/pDNA at the N/P ratio of 20 in medium with 50% FBS. **p* <0.05 when compared to the results at 0 h.