

Supporting Information

Ligand-functionalized degradable polyplexes formed by cationic poly(aspartic acid)s-grafted chitosan-cyclodextrin conjugates

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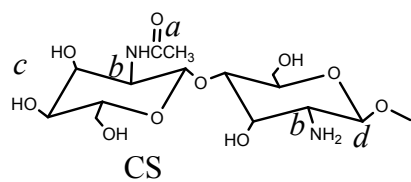
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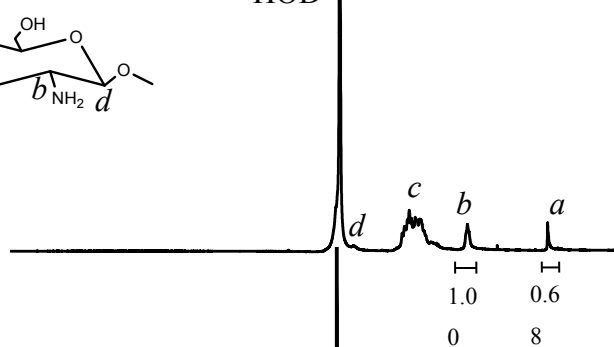
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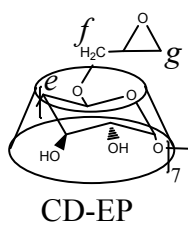
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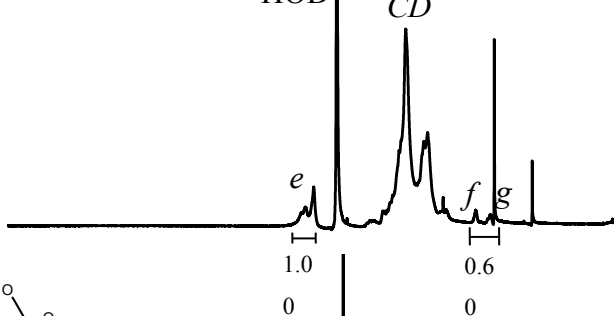
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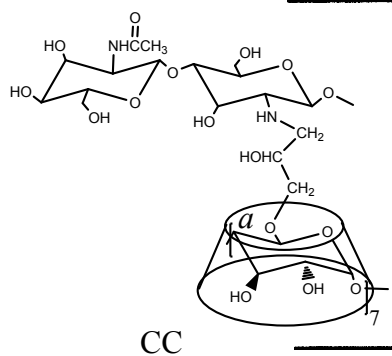
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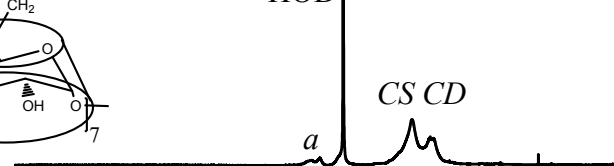
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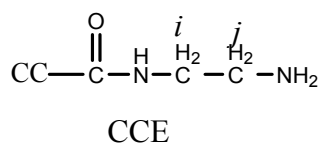
(c)



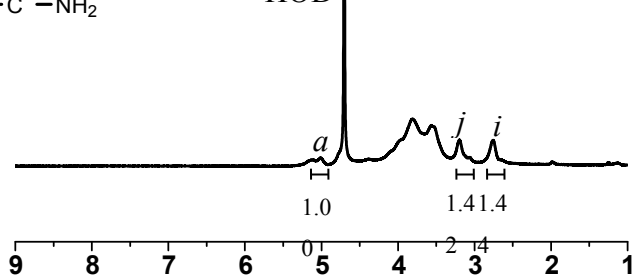
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(d)



HOD



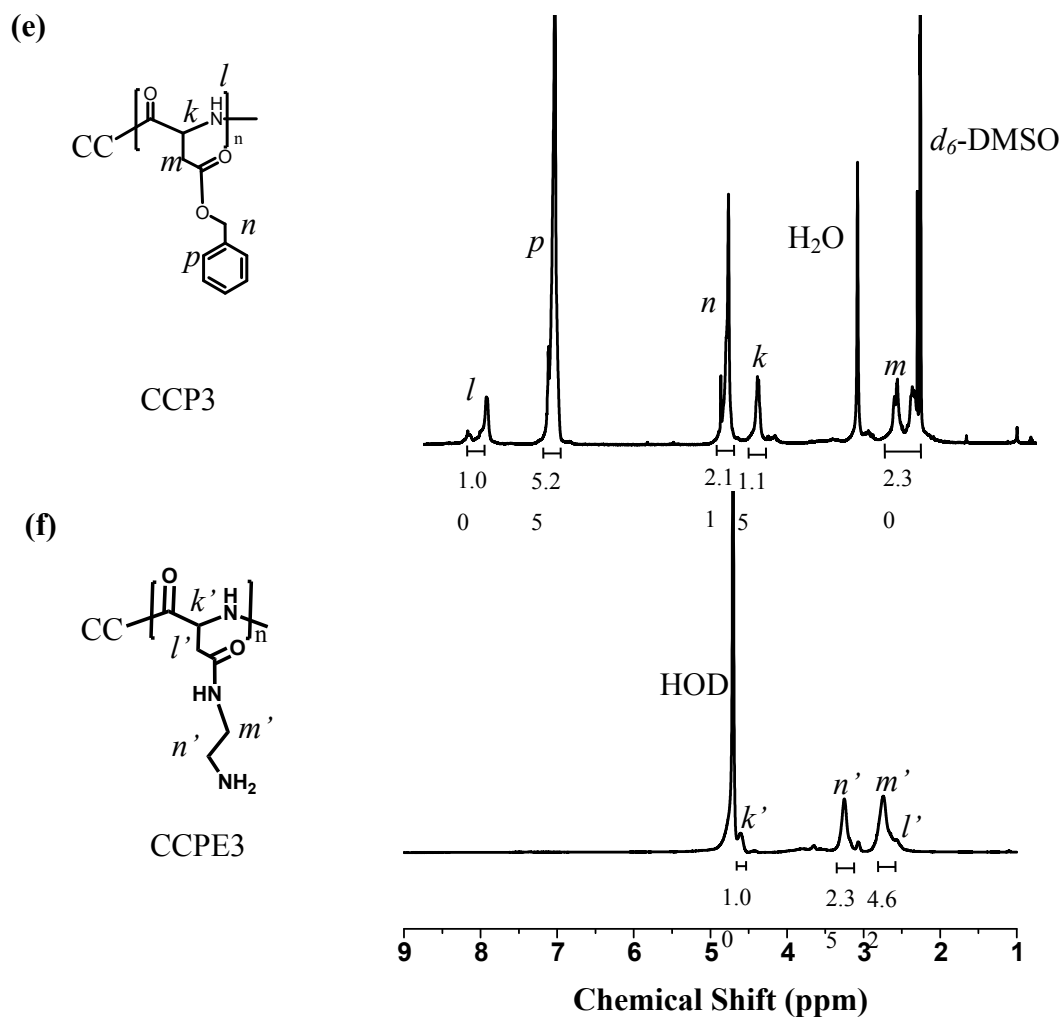
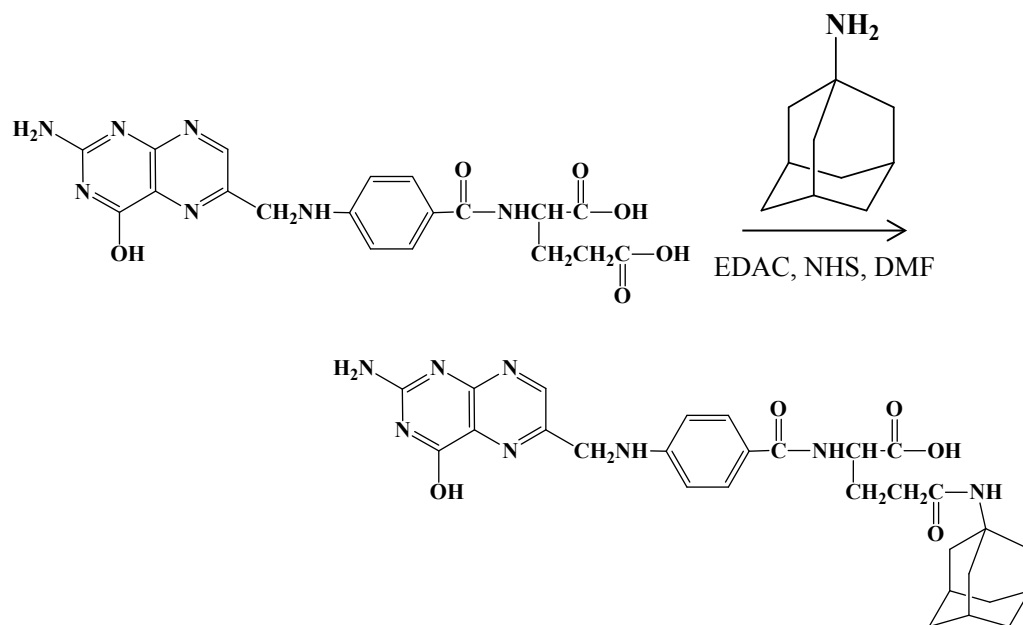


Figure S1. 400 MHz ^1H NMR spectra of (a) CS in D_2O , (b) CD-EP in D_2O , (c) CC in D_2O , (d) CCE in D_2O , (e) CCP3 in d_6 -DMSO, (f) CCPE3 in D_2O .

As shown in Figure S1, the chemical structures of the representative polymers synthesized in this work were characterized by ^1H NMR spectra. For CS, The peaks at δ 1.99 and 3.04 ppm were attributed to the methylene protons adjacent to amide (a, NH-CO-CH_3) and amino groups (b, CH-NH_2 , CH-NH(CO)CH_3), respectively. The number of the primary amino groups of one CS chain was about 16 based on the area ratio of peak a and peak b, as well as the molecular weight of CS (Table 1). The signals at δ 3.37-4.03 ppm were associated with the methyldyne and methylene protons (c, CH-O and $\text{CH}_2\text{-O}$) of saccharide units. The peak at δ 4.49 ppm was assigned to the methyldyne proton (d, O-CH-O). For CD-EP, the peaks from 3.42-4.41 ppm were attributed to the methyldyne and methylene protons (a, CH-O and $\text{CH}_2\text{-O}$) of glucose units. The peaks at about 5.0 ppm were associated with the unique methyldyne proton (e, O-CH-O) of glucose units. The signals at the chemical shifts of 2.63 and 2.82 ppm represented methylene protons (g, $\text{O-CH}_2\text{-CH}$ and f, $\text{O-CH}_2\text{-CH}$), respectively. Based on the integral area ratio of peak e and peaks f, g, about one

hydroxyl group on CD was functionalized by epichlorohydrin. Figure S1 (c) showed the ^1H NMR spectrum of CC. Both CS and CD were composed of glucose units, and the typical methylene protons adjacent to amide (NH-CO-CH_3) and amino groups ($\text{CH}_2\text{-NH}_2$) were not obvious. The peaks at 3.45-4.29 ppm were considered as the methyldyne and methylene protons of all the glucose units. For the CCE initiator obtained via the reaction of CC and ED, the peaks at 2.72 and 3.17 ppm were associated with methylene protons (i, $-\text{CO-CH}_2\text{-CH}_2$ and j, $-\text{CH}_2\text{-CH}_2\text{-NH}_2$). Based on the area ratio of peaks i, j and peak a (the typical signal of CDs of CC), as well as the molecular weight of CC (Table 1), the number of the ED-functionalized hydroxyl groups of one CCE chain was about 20. Due to the possibility of the reaction of hydroxyl groups of CS with ED, it is very difficult to exactly calculate the number of the ED-functionalized hydroxyl groups per CD unit. Based on the molecular weights of CS and CC (Table 1), about four CD units were tied on one CS chain, indicating that about 12 (=16-4) primary amine groups of CS remains. Thus, one CCE initiator possessed about 32 (=20+12) primary amine groups for the post ring-opening polymerization. In Figure S1(e), the signals at δ 4.89, 8.13, 2.76 and 5.04 ppm represented the methyldyne protons of $\text{CO-CH(CH}_2\text{)-NH}$ (k), amino protons of $\text{CO-CH(CH}_2\text{)-NH}$ (l), methylene protons of $\text{CH-CH}_2\text{-CO}$ (m) and methylene protons $\text{C-CH}_2\text{-O}$ (n), respectively. After aminolysis reaction, the typical chemical shifts p ($\delta = 7.28$ ppm) of CCP3 (as shown in Figure S1(e)) associated with benzyl ester pendant groups disappeared in CCPE3 (Figure S1(f)). The new chemical shifts δ 2.68 and 3.24 ppm were assigned to the structure (m', C(O)-NH-CH_2) and (n', $\text{CH}_2\text{-CH}_2\text{-NH}_2$), respectively.



Scheme S1. Synthetic processes of Ad-modified FA (Ad-FA).

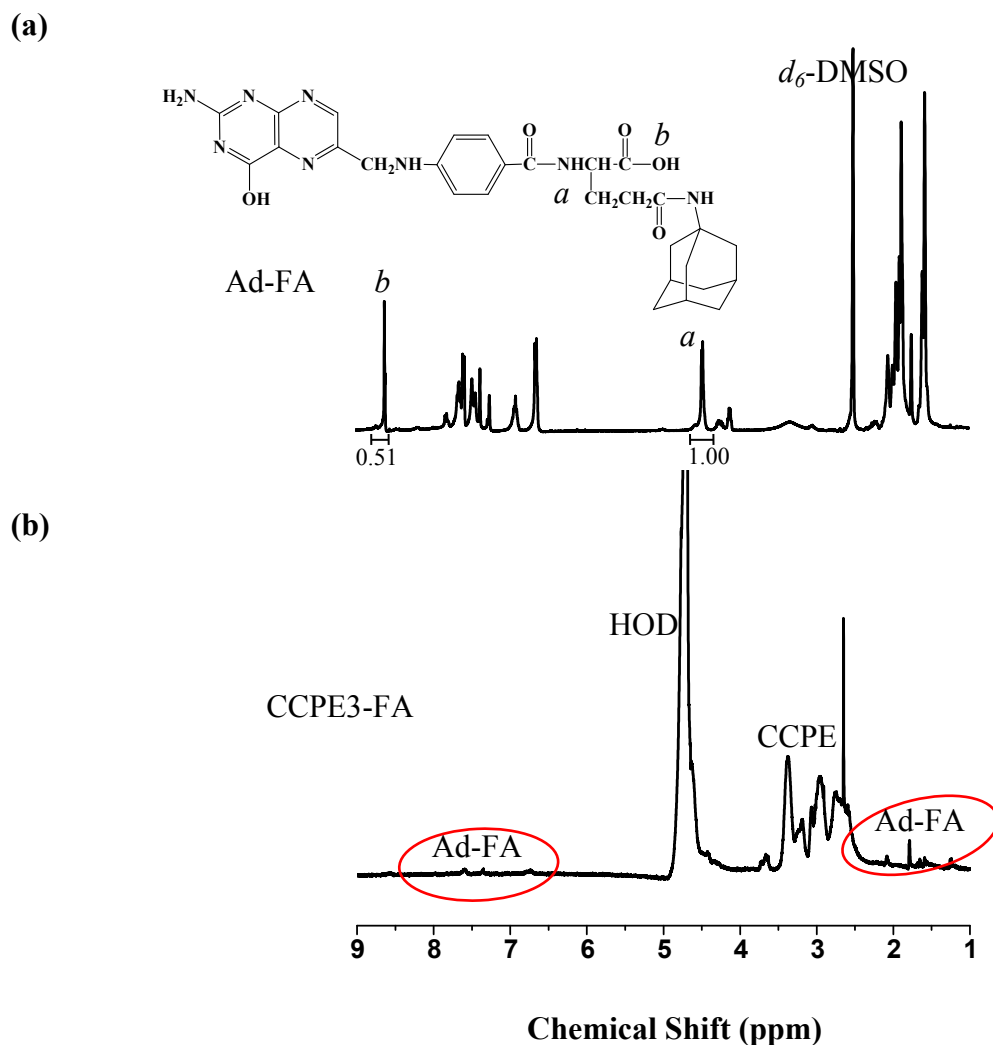


Figure S2. 400 MHz ^1H NMR spectra of (a) Ad-FA in d_6 -DMSO and (b) CCPE3-FA in D_2O .

Figure S2 showed the ^1H NMR spectra of Ad-FA and CCPE3-FA. For FA, the area of peak b (8.66 ppm, $-\text{CO}-\underline{\text{O}}\text{H}$) should equal to the area of peak a (4.52 ppm, $-\text{NHCH}-\underline{\text{C}}\text{H}_2-\text{C}\text{H}_2-$). As shown in Figure S2(a), after reacting with Ad, one carboxyl group of FA disappeared, and the area of peak b decreased by half. Such result confirmed the successful preparation of Ad-FA. For CCPE3-FA prepared via the host-guest interaction of CCPE3 and Ad-FA (Figure S2(b)), the weak signals at 6.89-8.34 ppm and 1.45-2.0 ppm were attributed to Ad-FA, indicating that FA-functionalized CCPE was successfully obtained.

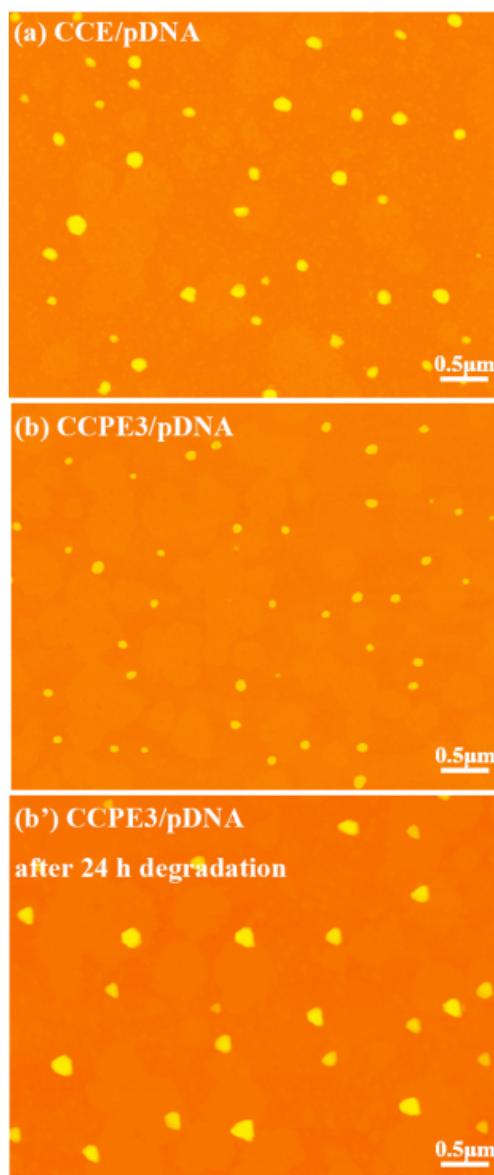


Figure S3. AFM images of (a) CCE/pDNA and (b, b') CCPE3/pDNA polyplexes before and after 24 h degradation at the N/P ratio of 20.

After 24 h degradation, the size of CCPE3/pDNA polyplexes became larger (Figure S3b'). This may be caused by decreased condensation ability of increasing oligomers generated after degradation. The decreased higher-molecular-weight portions of CCPE3 could not efficiently condense pDNA and led to loose conformation of polyplexes with larger size.

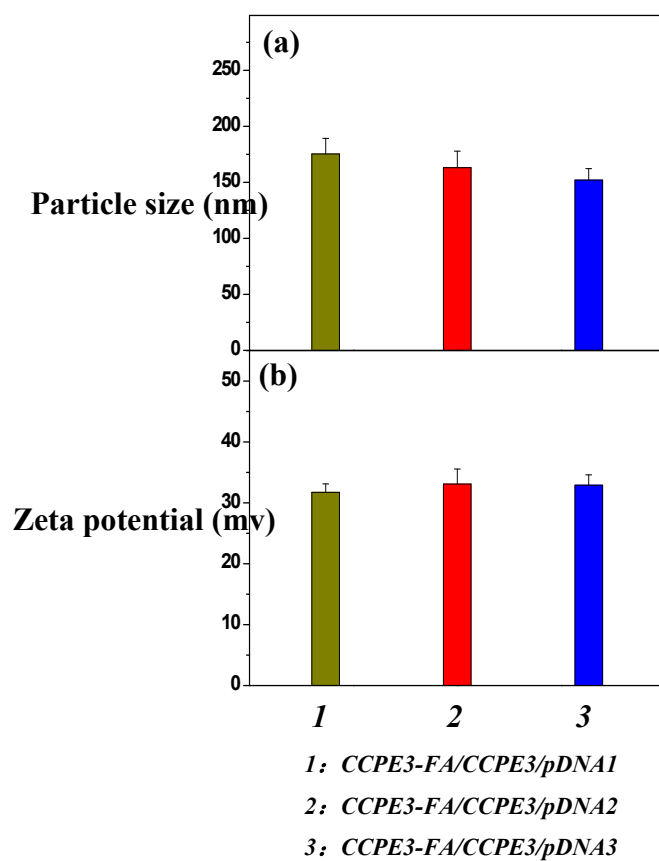


Figure S4. Particle sizes (a) and zeta potentials (b) of the different CCPE3-FA/CCPE3/pDNA complexes at the final N/P ratio of 20.

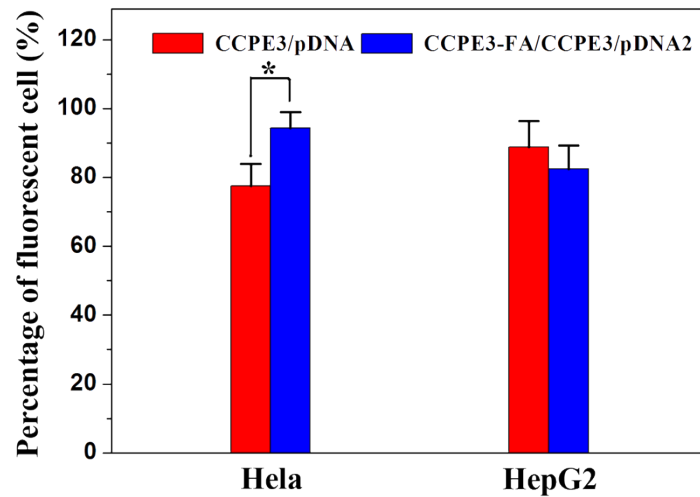


Figure S5. Cellular uptake efficiencies where the HeLa and HepG2 cells were treated with CCPE3/pDNA and CCPE3-FA/CCPE3/pDNA2 polyplexes for 4 h at the N/P ratio of 20. The cellular uptake efficiency of the control HeLa and HepG2 cells without treatment was negligible (about 2.3%). Error bars represent the standard deviation (mean \pm SD, n=3, *p <0.05).