Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Polymeric Assembly of Hyperbranched Building Blocks to Establish Tunable Nanoplatforms for Lysosome-Acidity-Responsive Gene/Drug Co-delivery

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Optimization of HBPO/OEI600-PBA ratio in the assembly based on *in vitro* luciferase assay

To simplify the study, the optimal molar ratio of HBPO *versus* OEI600-PBA was first established on the basis of *in vitro* luciferase assay, where the feeding amount of OEI600-PBA was fixed ($w_{OEI600\text{-}PBA}/w_{pGL\text{-}3}$ = 20 : 1) while that of HBPO was varied. As shown in Figure S1A, the best outcome was achieved at the molar ratio of 0.1: 1. In the case, periphery clustering of OEI600-PBA onto HBPO functions like merging a couple of OEI600-PBA together. The assembly led to the considerably promoted charge density, favoring the improved transfection. Theoretically, further addition of HBPO would decrease the average OEI600-PBA amount per nanoassembly and consequently impair the transfection efficacy. This can find the supporting evidence by the finding that the zeta potential of the nanoassemblies peaked at the same ratio of 0.1: 1 detected for the optimal transfection (Figure S1B). In fact, there even appeared the precipitates in the case of higher HBPO usage, likely due to the excessively reduced hydrophilicity/hydrophobicity balance ratio. Based on these data, we therefore employed HBPO(OEI600-PBA)$_{10}$ nanoassembly (molar ratio of HBPO *versus* OEI600-PBA = 1 : 10) for the experiments throughout the study.

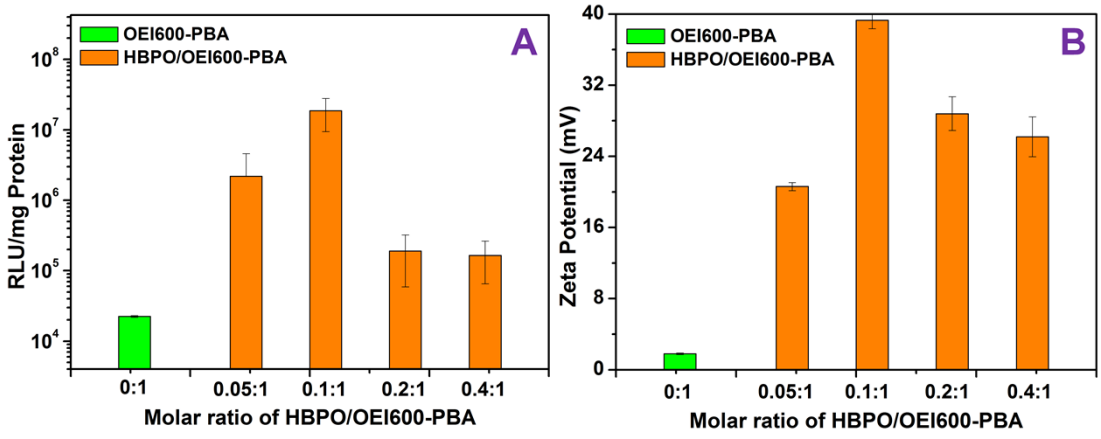

Fig. S1. (A) Transfection efficiency by luciferase assay of HBPO/OEI600-PBA/pGL-3

prepared with different molar ratios (HBPO *versus* OEI600-PBA) in 293T cells. For the HBPO/OEI600-PBA/pGL-3complex, the weight ratio of OEI600-PBA versus pGL-3 was fixed its optimal transfection ratio of 20 : 1. The concentration of pGL-3 plasmid was fixed at 1 μg/mL. ; (B) Zeta potential of HBPO/OEI600-PBA assemblies with varied molar ratio of HBPO *versus* OEI600-PBA. Data were shown as mean ± SD (n = 3).

Size of OEI600-PBA/pGL-3 and HBPO(OEI600-PBA)$_{10}$/pGL-3 polyplexes

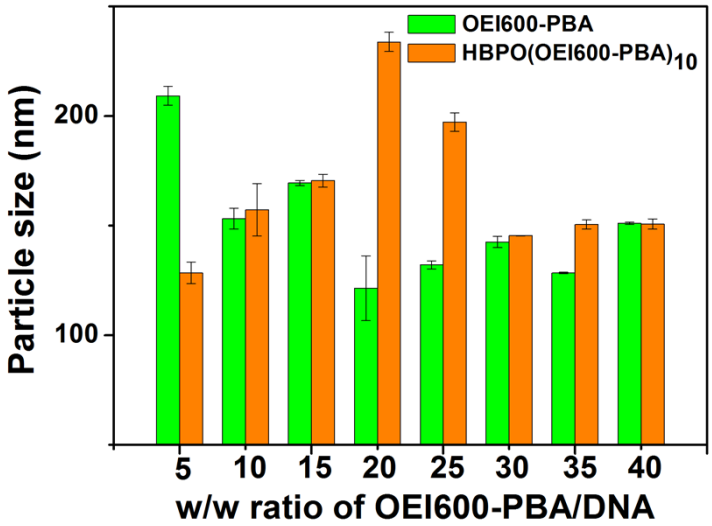

Fig. S2. Mean particle size of OEI600-PBA/pGL-3 and HBPO(OEI600-PBA)$_{10}$/pGL-3 polyplexes prepared at different $w_{OEI600\text{-}PBA}/w_{pGL\text{-}3}$ ratios. The comparison between OEI600-PBA and HBPO(OEI600-PBA)$_{10}$ was based on the condition that the OEI600-PBA/DNA feed ratio was identical. Data were shown as mean ± SD (n = 3).

Observation on blank or OEI600-PBA containing buffer solution upon addition of Nile Red dye (NRD)

After NRD was introduced into the blank or OEI600-PBA containing buffer solution, lots of red deposits appeared regardless of pH variation in both cases (Fig. S3). With the identical treatment, in contrast, the HBPO(OEI600-PBA)$_{10}$ solution remained always translucent at pH 7.4 with no detection of deposits, and the color faded to almost colorless (Fig. 3A).

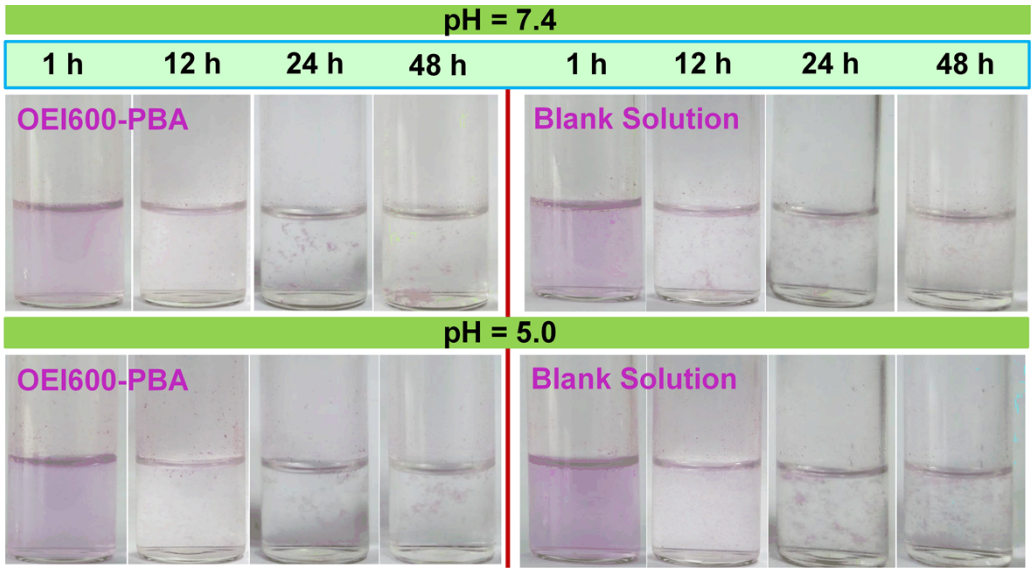

Figure S3. Variation of blank (Right) and OEI600-PBA (Left) buffer solution (2 mL) at different intervals after the introduction of Nile Red dye solution in THF (0.01mL, 0.06 mg/mL). The experiment was conducted in an open vessel at 30 ^{o}C to allow the solvent evaporation. The pH value of the buffer was 7.4 in PBS (upper) and 5.0 in acetate buffer solution (lower), respectively.

CLSM images of HeLa cells after 4-h co-incubation with drug loaded HBPO(OEI600-PBA)$_{10}$ nanoassembly

The cellular uptake efficiency of CUR or DOX loaded HBPO(OEI600-PBA)$_{10}$ nanoassembly in HeLa cells was estimated by CLSM. As shown in Fig. S4, green fluorescence of CUR or red fluorescence of DOX was distributed evenly in the cytoplasm area of HeLa cells after 4-h co-incubation with CUR or DOX loaded HBPO(OEI600-PBA)$_{10}$.

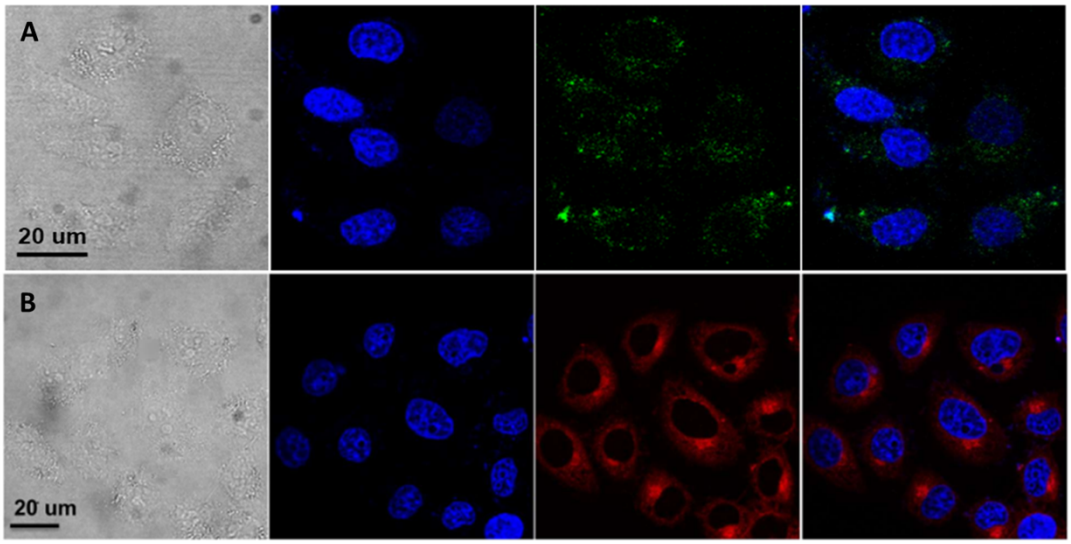

Fig. S4. CLSM images of HeLa cells after 4-h incubation with CUR (A) and DOX (B) loaded HBPO(OEI600-PBA)$_{10}$ nanoassembly, respectively. Nuclei were stained blue by Hoechst 33342.

^{1}H NMR spectrum of OEI600-PBA

The chemical structure of OEI600-PBA was verified by ^{1}H NMR spectrometry as shown below. The characteristic NMR resonances belonging to OEI600 and phenyl group of PBA can be distinctly detectable. The signals at 3.9 ppm were attributed to the methylene protons adjacent to phenyl group and those appearing at 3-2 ppm were ascribed to the protons of OEI600.

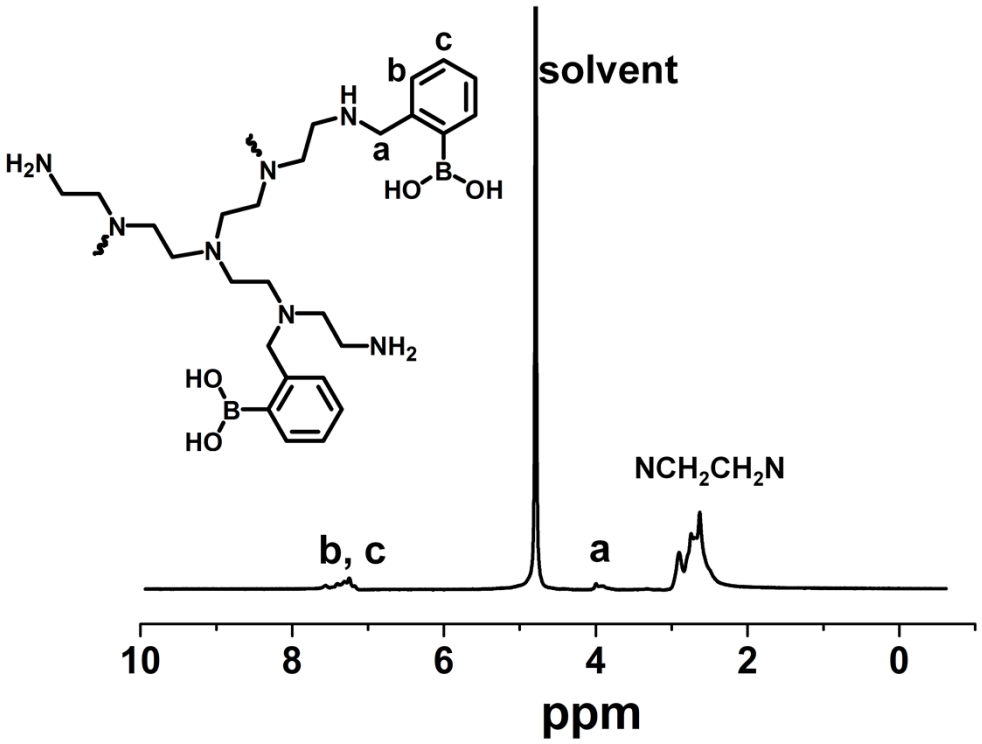

Fig. S5. ^{1}H NMR spectrum of OEI600-PBA in D_2O.

Cytotoxicity of free DOX and DOX-loaded nanoassembly in HeLa cells

The cytotoxicity of free DOX and DOX-loaded nanoassembly in HeLa cells was determined by MTT assay. As shown in Fig. S6, DOX-loaded nanoassembly showed a relatively lower cytotoxicity than free DOX, more possibly due to the controlled release pattern of DOX in the former case and the easier cellular entry of free DOX through diffusion pathway.

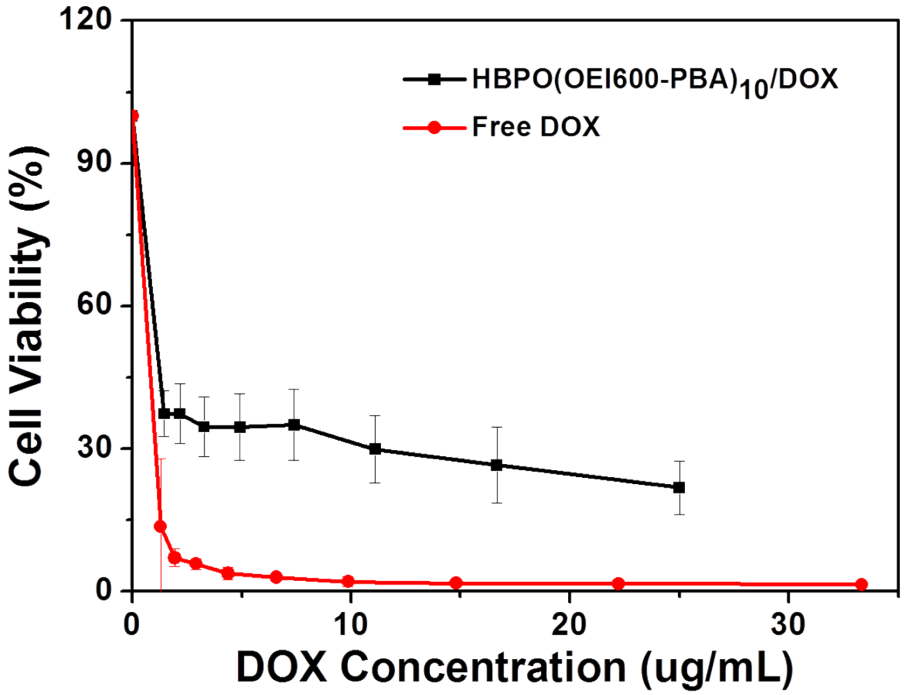

Fig. S6. Cell viability of HeLa cells after 48-h incubation with free DOX•HCl and DOX-loaded nanoassembly.