

**pH-sensitive oncolytic adenovirus hybrid targeting acidic tumor microenvironment and angiogenesis**

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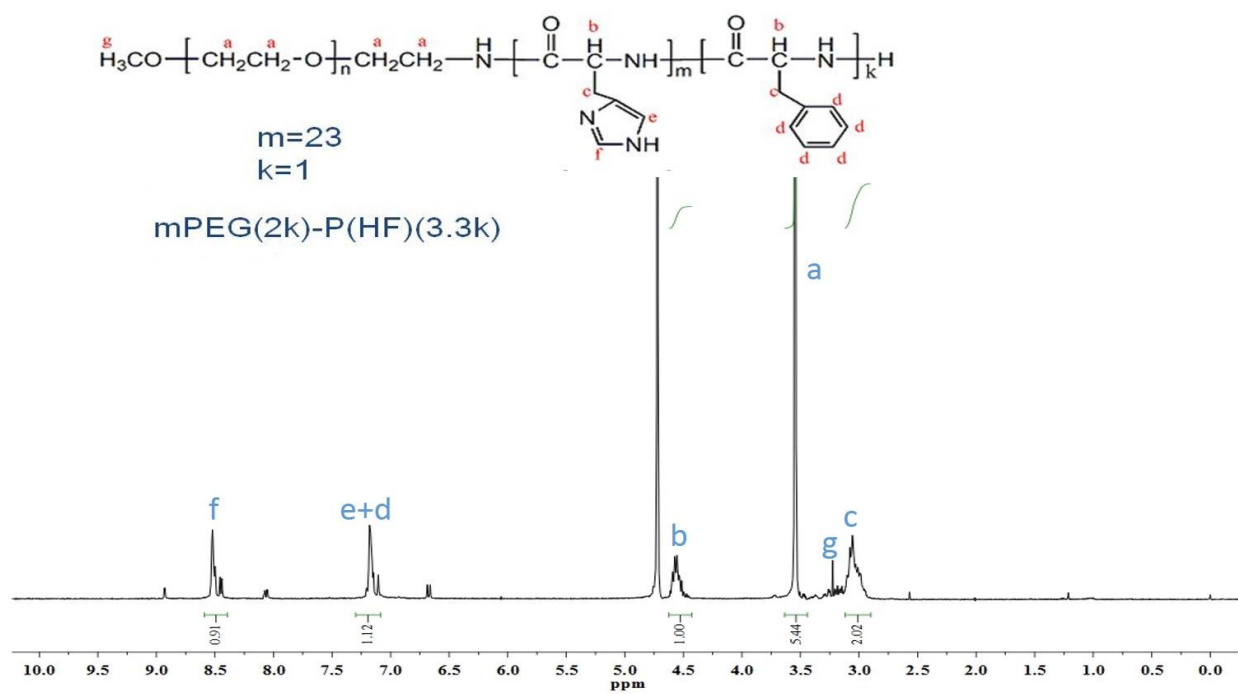
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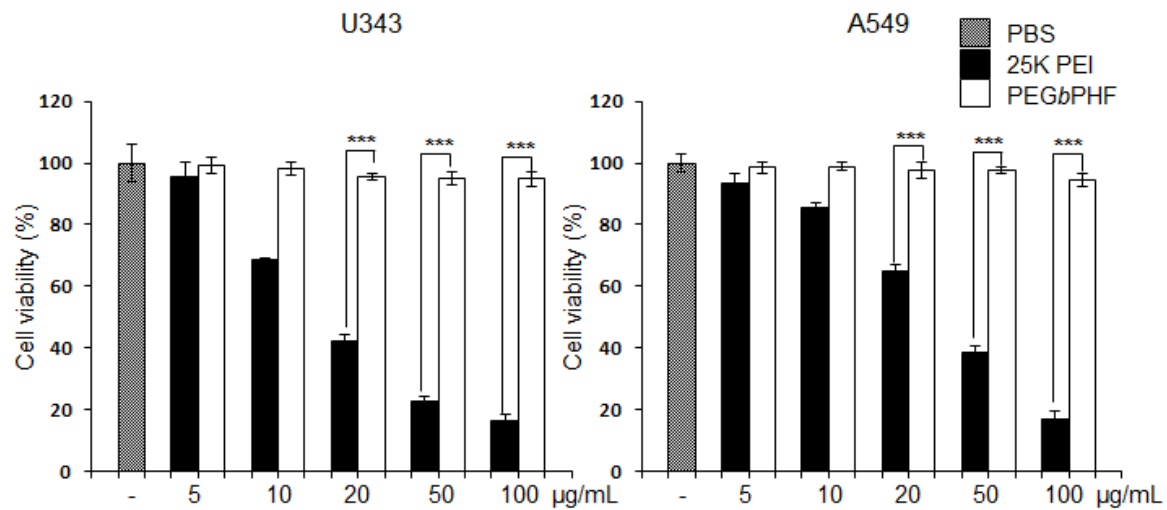
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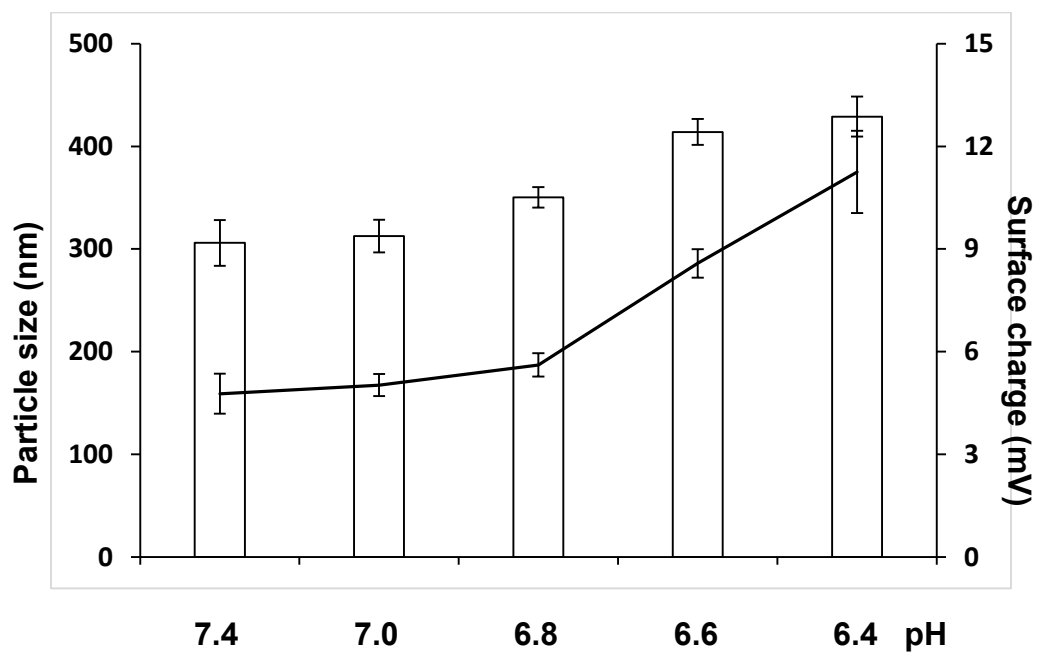
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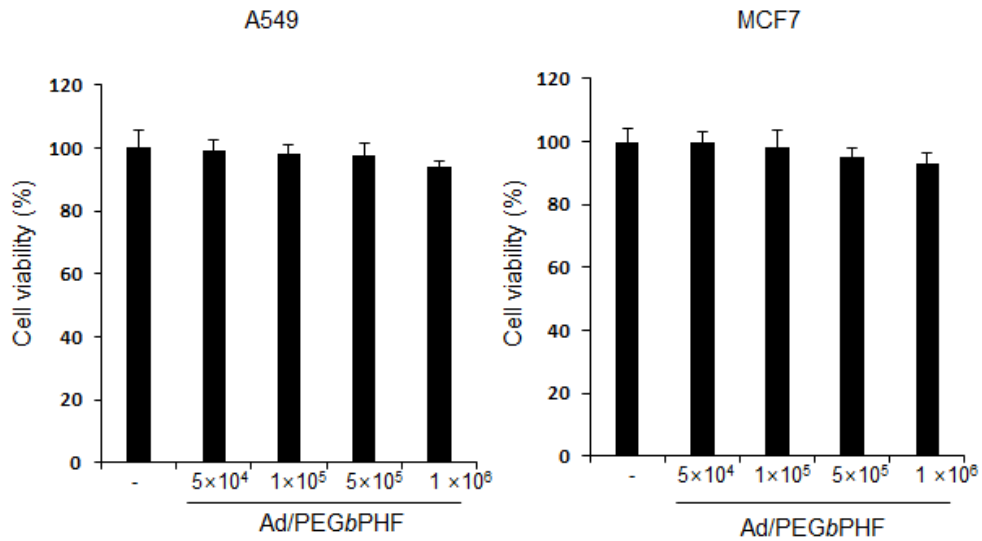
**Fig. S1.**  $^1\text{H}$  NMR spectrum of PEGbPHF in  $\text{D}_2\text{O}/\text{DCI}$  (1mL  $\text{D}_2\text{O}$ +20 $\mu\text{L}$  DCI) at 25  $^\circ\text{C}$ .



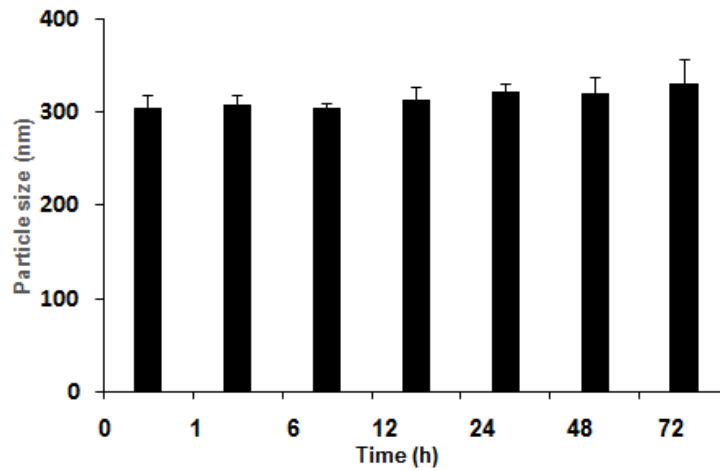
**Fig. S2.** Effect of 25K PEI and PEGbPHF on cell viability. U343 and A549 cells were treated with PBS, 25K PEI, or PEGbPHF, followed by an MTT assay at 48 h post treatment. Results were normalized against the negative control (PBS). Each value represents mean  $\pm$  SD of three separate experiments (n = 3 per experiment). \*\*\* $P < 0.001$  versus 25K PEI.



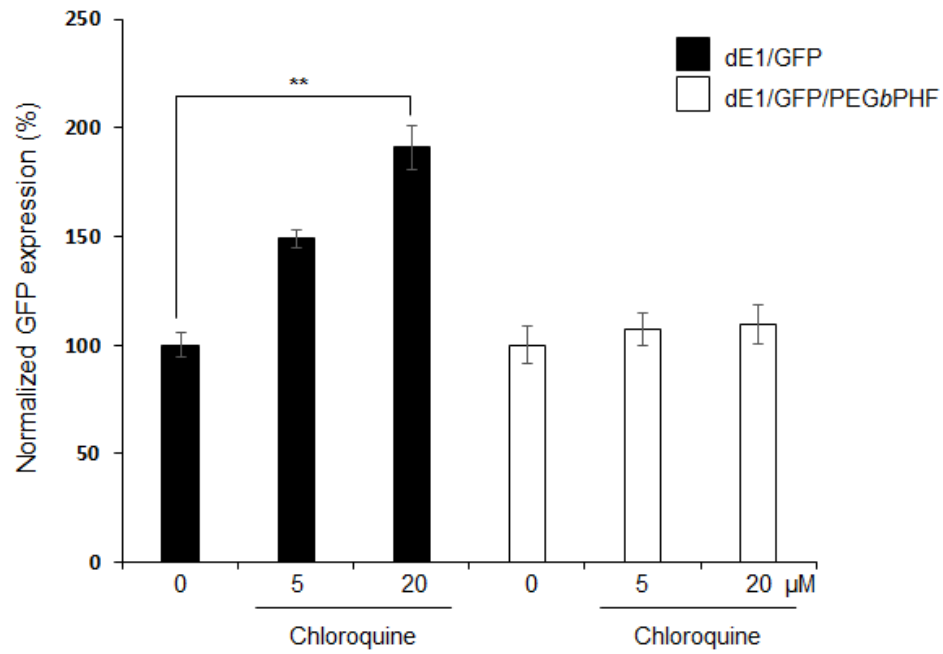
**Fig. S3.** Particle size and zeta-potential as a function of pH (7.4–6.4). The average size (nm) and zeta-potential value (mV) of dE1/GFP ( $1 \times 10^{10}$  VP) were measured at various pH from 7.4 to 6.4. The data represent the means  $\pm$  SD for three replicates.



**Fig. S4.** Effect of Ad/PEGbPHF complex on cell viability. A549 and MCF7 cells were treated with various Ad/PEGbPHF ratios, followed by an MTT assay at 48 h post treatment. Results were normalized against the negative control (PBS). Each value represents mean  $\pm$  SD of three separate experiments (n = 3 per experiment).



**Fig. S5.** Stability evaluation of dE1/GFP/PEGbPHF. dE1/GFP/PEGbPHF (1:  $1 \times 10^6$  ratio) complex was dispersed in phosphate saline buffer (PBS) at room temperature, and the average size of Ad/PEGbPHF was measured at pre-determined time intervals from 0 to 72 h. The data represent the means  $\pm$  SD for three replicates



**Fig. S6.** Transduction efficiency of naked dE1/GFP and dE1/GFP/PEGbPHF (1: 1 x 10<sup>6</sup> ratio) in the presence or absence of chloroquine (CQ) in A549 cells. Relative transduction efficiency: experimental values / control values. The data represent the means ± SD for three replicates