Electronic Supplementary Information

Ketalized Poly(amino ester) for Stimuli-Responsive and Biocompatible Gene Delivery

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1. Characterization of K-PAE by ^{1}H NMR spectroscopy

Structure of K-PAE was confirmed by the peaks for methylene protons of diaminoethane (e, f, i, and j) and the peaks for methylene protons of ethylene glycol (peak: d, o, and p) in ^{1}H NMR spectrum (Fig. S1). The peak for methylene protons adjacent to the ester (peak: c) and the sharp singlet peak for the methyl protons of ketal linkage (peak: q) represent the stimuli-cleavable structure of K-PAE and were used to qualify the hydrolysis kinetics under various conditions.

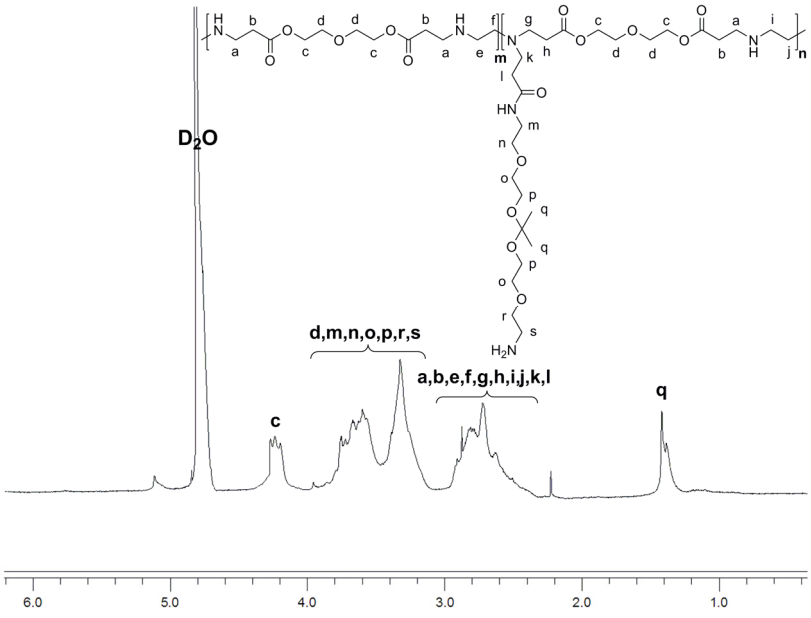

Fig. S1. ^{1}H NMR spectrum of K-PAE.

2. Transfection efficiency and cytotoxicity of DNA/K-PAE polyplexes at various N/P ratios

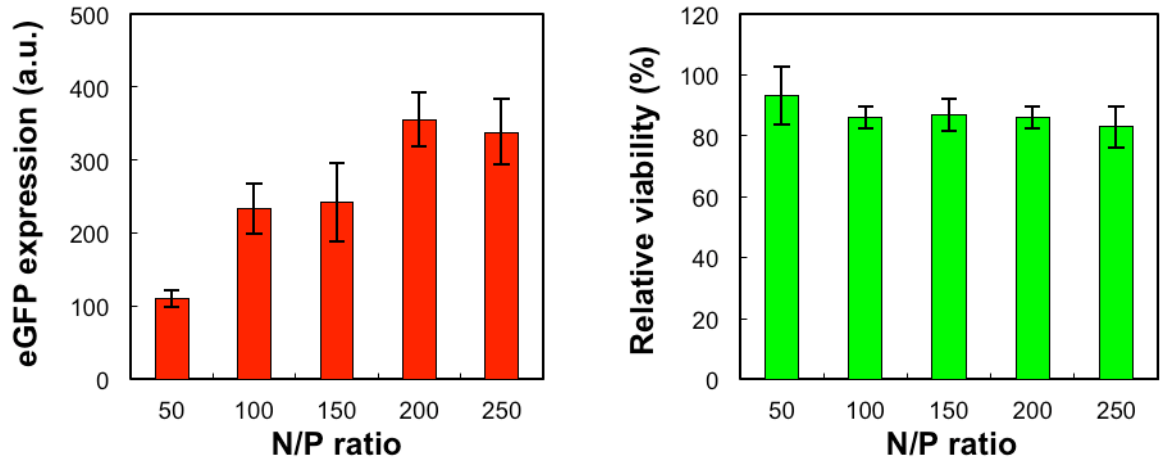

Fig. S2. eGFP expression and relative viability of NIH 3T3 cells incubated with DNA/K-PAE polyplexes at various N/P ratios. The highest gene expression by DNA/K-PAE polyplexes was obtained at an N/P ratio of 200 where the most efficient DNA condensation was observed (Fig. 1).

3. Cellular uptake of DNA/K-PAE and DNA/NK-PAE polyplexes

1 μg of Alexa Fluor 488-labeled DNA (AF488-DNA) was mixed with K-PAE or non-ketalized poly(amino ester) (NK-PAE) in DI water (50 μL in total) at their optimum N/P ratios of 200. NIH 3T3 cells were seeded in a 24-well plate (5×10^4 cells/well), 24 h before transfection. Each polyplex solution was added to the cells with 250 μL of FBS-free DMEM. After the cells were incubated for 4 h at 37 °C and harvested, the fluorescence intensity of AF488-DNA in the cell was quantified by a Guava EasyCyte Plus flow cytometer.

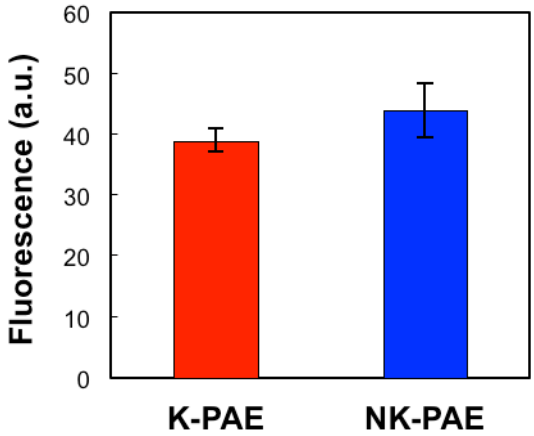

Fig. S3. Cellular uptake of AF488-DNA/K-PAE and AF488-DNA/NK-PAE polyplexes by NIH 3T3 cells. DNA/K-PAE polyplexes were internalized slightly less efficiently than DNA/NK-PAE polyplexes, due to lower DNA complexation efficiency.

4. Effect of ketalization ratios on transfection efficiency

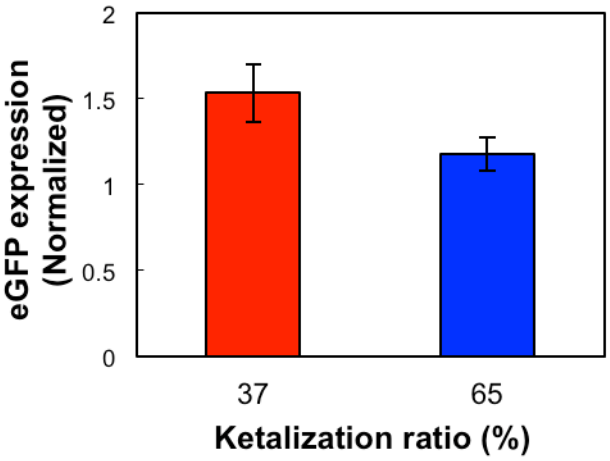

Fig. S4. Ketalization effects on transfection by DNA/K-PAE polyplexes. DNA was complexed with 37 and 65% ketalized K-PAE at N/P ratios of 200 and 280 (determined by the maximum transfection), respectively. 65% ketalized K-PAE was prepared by conjugating 3 equivalent of Cbz-protected amino ketal acrylamide (compound 6 in Scheme 1) with 1 equivalent of amino groups in poly(amino ester). eGFP expression in NIH 3T3 cells that were transfected by the polyplexes of DNA and differentially ketalized K-PAE was normalized by the transfection efficiency of NK-PAE.

5. Intracellular disassembly of DNA/NK-PAE polyplexes

NIH 3T3 cells were seeded at a density of 2×10^4 cells/well in a Falcon 8-well, 24 h prior to transfection. NK-PAE complexing Alexa Fluor 488 labeled-DNA in 500 μL of DI water at the optimum N/P ratio of 200 was further labeled with Alexa Fluor 555 dye and purified by size-exclusion column chromatography. 250 μL of polyplex solution in DMEM (0.5 μg of the labeled DNA) was added to the cells and incubated for 8 h. After the cells were fixed and nuclei-counter-stained, they were observed by confocal microscopy.

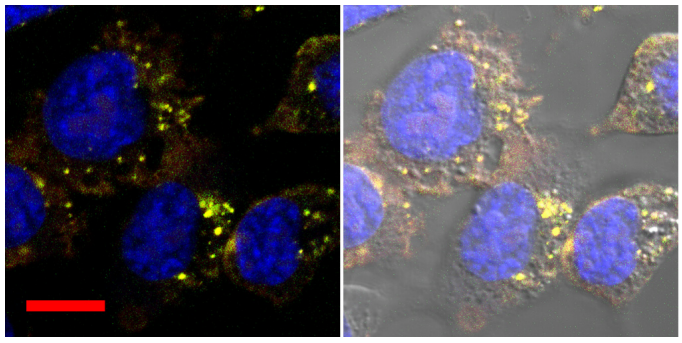

Fig. S5. Fluorescence and merged DIC/fluorescence micrographs of NIH 3T3 cells incubated with DNA/NK-PAE polyplexes for 8 h. (DNA: greed dots; polymer: red dots; co-localized DNA and polymer: yellow dots). Scale bar = 10 μm.