Electronic Supplementary Information (ESI)

Zinc-Chelated Imidazole Groups for DNA Polyion Complex Formation

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Experimental details:

Materials. 1-Vinylimidazole (VIm) was purchased from Aldrich Chemical Co. (Milwaukee, WI). The VIm was distilled under reduced pressure. 2,2'-Azobis(2,4-dimethyl-valeronitrile) (V-65) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and the V-65 was recrystallized from ethanol. Zinc chloride was from Kanto Chemical Co., Inc. (Tokyo, Japan). Poly(ethylenimine) (PEI) solution (M_n=~60,000) was from Sigma Chemical Co. (St. Louis, MO). All other chemicals of a special grade were used without further purification.

Preparation of PVIm-Zn. VIm (300 μL) and V-65 (15.8 mg) as an initiator were dissolved in 2.7 mL *N*,*N*-dimethylformamide (DMF). Radical polymerization reaction was carried out at 45 °C for 1 day. After the reaction, the content was poured into a large excess of acetone, and the precipitate was dried *in vacuo*. The number-average molecular weight of the isolated polymer determined by gel filtration chromatography was about 6.9×10^3. The ^{1}H NMR spectrum of the resulting polymer showed the characteristic signals [δ 1.8-2.2 (methylene), 2.3-3.7 (methine),

6.4-7.2 (imidazole) ppm].

The resulting polymer PVIm (50 mg) and zinc chloride (181.28 mg) were dissolved in 100 mL of H_2O (deionized water) and incubated at room temperature for 1 day. The supernatant was dialyzed against distilled water using a Spectra/Por 7 membrane (molecular weight cutoff = 10^3), to remove free zinc ions and chloride ions, followed by the centrifugation (30 min, 5000 rpm). The resulting supernatant was used as a stock solution. The zinc in the stock solution was determined by atomic absorption spectrometry at 213.9 nm using a Varian SpectrAA-640 (Varian Australia Pty., Ltd., Australia). The PVIm in the stock solution was then determined by weighing the freeze-dried sample of the stock solution, followed by the subtraction of the zinc component.

pH-Dependent Chelation Assay. To 270 μL of 10 mM sodium phosphate buffer (pH 7.4 or pH 5.0) containing 130 mM NaCl was added a 30 μL of the solution of the PVIm-Zn, and the resulting solution was incubated at room temperature for 2 h. Then, the PVIm-Zn solution was centrifuged at 500 rpm for 20 min using a centrifugal filter unit (Amicon® Ultra, Millipore Co., Billerica, MA) with a 3000 molecular weight cutoff to collect the released Zn^{2+} ions. The Zn^{2+} ions released from the PVIm-Zn was finally determined by atomic absorption spectrometry at 213.9 nm using a Varian SpectrAA-640 (Varian Australia Pty., Ltd., Australia).

Agarose Gel Retardation Assay. Plasmid DNA (pGL3-Control Vector; from Promega Co.) was dissolved in TE buffer, and the solution was diluted with 50 mM sodium phosphate buffer (pH 7.4). To the resulting plasmid DNA solution was added the PVIm-Zn solutions in the 50 mM sodium phosphate buffer at various PVIm-Zn/DNA ratios (+/- = 2-40). The +/- ratio means the

ratio of the number of the cation of the Zn^{2+} ions in PVIm-Zn to the number of the anion of the phosphate groups in DNA. The number of the Zn^{2+} cation was determined by atomic absorption spectrometry, and the number of the phosphate anion was determined by UV spectrometry. After 30 min of incubation at room temperature, each sample (corresponding to 200 ng of DNA) was mixed with a loading buffer and loaded onto a 1% agarose gel containing 1 μg/mL of ethidium bromide. Gel electrophoresis was run at room temperature in 50 mM sodium phosphate buffer (pH 7.4) at 50 V for 15 min. The DNA bands were visualized under UV irradiation.

Cell Viability Assay. HepG2 cells (a gift from the Japan Health Sciences Foundation), human hepatoma cell line, were cultured in tissue culture flasks containing Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated FBS. The cells were seeded at 1×10^4 cells/well in a 96-well plate and incubated overnight at 37 °C in a 5% CO_2 incubator. The cells were treated with PVIm-Zn/DNA (+/- = 1-8) or PEI/DNA (+/- = 2, 10) complexes under the same conditions for the transfection procedure (as below) and incubated for 48 h at 37 °C. By further incubation for 4 h, the cell viability was measured using the Alamar Blue assay[1] in triplicate.

Transfection Procedure. In a typical 96-well plate experiment, 1×10^4 cells/well HepG2 cells were transfected in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated FBS by the addition of 15 μL of PBS (-) containing 200 ng of plasmid DNA encoding the modified firefly luciferase (pGL3-Control Vector) and complexed with PVIm-Zn (+/- = 1-8) or PEI (+/- = 2, 10). After 1 day of incubation, the medium was removed and the cells were further incubated for 2 days in the Dulbecco's modified Eagle's medium supplemented with 10%

FBS. Then, the cells were subjected to the luciferase assay (Promega kit) according to the manufacturer instruction. Luciferase activities were normalized by protein concentrations and are presented as relative light unit (RLU). Protein concentrations were determined by BCA protein assay kit (Pierce) according to the manufacturer instruction.

Reference:

1 J.M. Unsworth, F.R.A.J. Rose, E. Wright, C.A. Scotchford and K.M. Shakesheff, *J. Biomed. Mater. Res.*, 2003, **53**, 617.

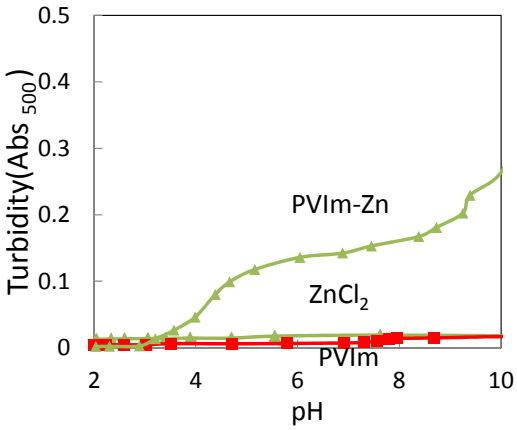

Fig. S1. Effect of pH on the solution turbidity in water: PVIm (3.3 mg/mL), $ZnCl_2$ (6.0 mg/mL), PVIm-Zn (PVIm, 3.3 mg/mL; $ZnCl_2$, 6.0 mg/mL). The turbidity was measured by monitoring the absorbance at 500 nm of the aqueous solution during the acid-base titration.

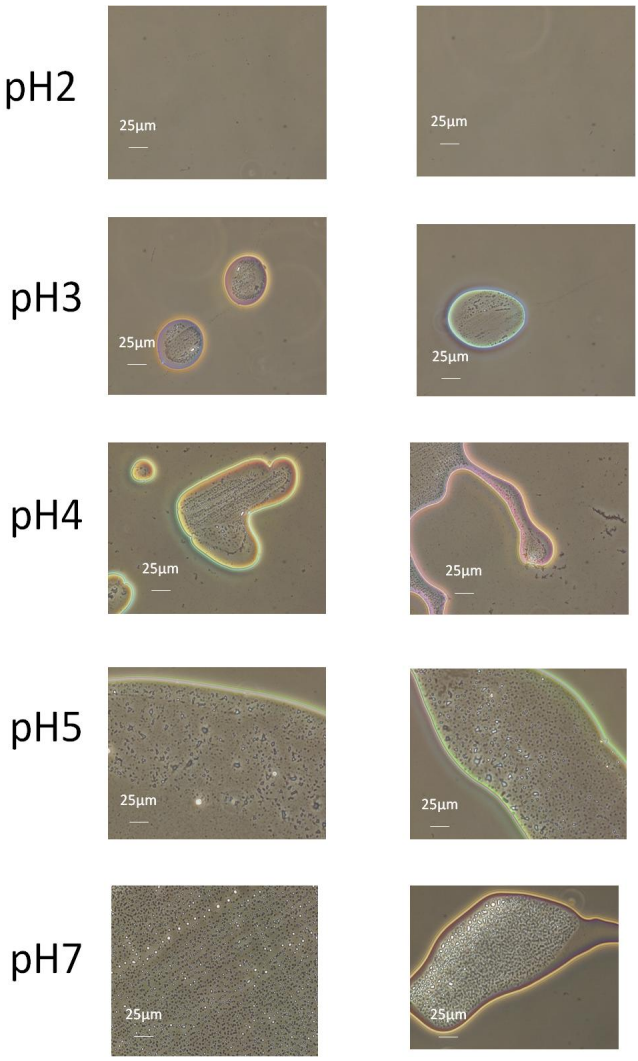

Fig. S2. Morphology of the PVIm-Zn complexes observed by phase contrast microscope under various pH conditions.

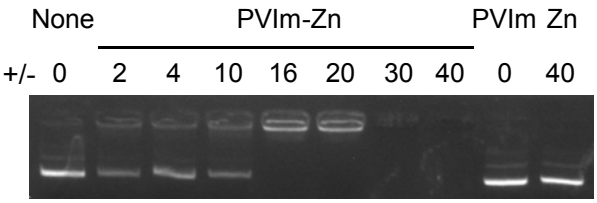

Fig. S3. Magnification of Fig. 2A.

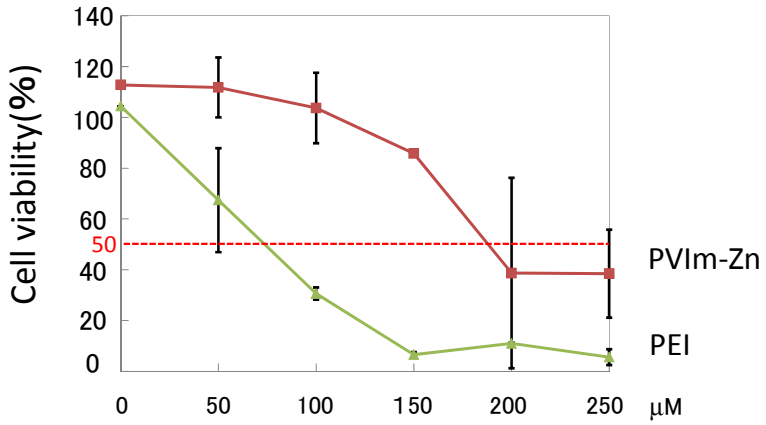

Fig. S4. IC_{50} of PVIm-Zn compared with PEI.