Supporting Information

Synergistic effects of hyperosmotic polymannitol based non-viral vectors and nanotopographical cues for enhanced gene delivery

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Experimental

Fabrication of nanopatterns

Silicon wafers were spin-coated with a photoresist (Shipley, Marlborough, MA) and then nanopatterned via electron-beam lithography (JBX-9300FS, JEOL). After photoresist development (MF320, Shipley), exposed silicon was deep reactive ion etched (STS ICP Etcher) to form arrays. The remaining photoresist on silicon wafers was removed using ashing process (BMR ICP PR Asher) and then diced into silicon masters for subsequent replica molding.

To fabricate topographic nanopattern arrays, poly(urethane acrylate) (PUA) was used as a mold material from the silicon master. Briefly, a UV-curable PUA precursor (Minuta Tech., South Korea) was drop-dispensed onto the master and brought into contact with a 100 μm-thick polyethylene terephthalate (PET) film (SKC Inc., South Korea) as a backing plane. After subsequent irradiation of UV for few tens of seconds, a negative PUA replica was formed on the PET film.

Regularly spaced ridge and groove with the different widths were replicated from the prefabricated master PUA molds over a large area of 25 × 25 mm^2. Prior to application of the PUA mold, the glass substrate was cleaned with isopropyl alcohol (IPA), thoroughly rinsed in distilled ionized water, and then dried in a stream of nitrogen. Subsequently, an adhesive agent (phosphoric acrylate: propylene glycol monomethyl ether acetate = 1:10, volume ratio) was spin-coated to form a thin layer (~100 nm) for 30 s at 3000 rpm. A small amount of the same PUA precursor was drop-dispensed on the substrate and a PUA mold was directly placed onto the surface. The PUA precursor spontaneously filled the cavity of the mold by means of capillary action and was cured by exposure to UV light (λ = 250–400 nm) for ~30 s through the transparent backplane (dose = 100 mJ cm^{-2}). After curing, the mold was peeled off from the substrate using a sharp tweezer.

Synthesis of polymannitol based gene transporter (PMGT)

PMGT was synthesized in a two-step reaction in which mannitol was first esterified with methacryloyl chloride to form mannitol dimethacrylate (MDM) monomer, and then copolymerized with bPEI (1.2 kDa) by a Michael addition reaction to obtain PMGT. PMT was prepared by same method as previously reported[10].

Synthesis of MDM

Mannitol dimethacrylate (MDM) monomer was synthesized by reaction of mannitol with 2 equivalents of methacryloyl chloride. An emulsion was prepared by dissolving mannitol (1 g) in DMF (20 ml) and pyridine (10 ml) followed by drop wise addition of methacryloyl chloride solution (1.2 ml dissolved in 5ml DMF) at 4°Cwith

constant stirring overnight. After reaction completion, HCl.pyridine salts were filtered and filtrate was dropped to diethylether. The product was precipitated in a syrupy liquid form and dried over vacuum.

Synthesis of PMGT

PMGT was prepared by Michael addition reaction [15] between LMW bPEI and MDM. Briefly,the synthesized MDM (0.38 g) dissolved in DMSO (5 ml) was added drop wise to 1 equivalent of bPEI (1.2 kDa, dissolved in 10 ml DMSO) and reacted at at 60°C with constant stirring for 24 h. After reaction completion, mixture was dialyzed using a Spectra/Por membrane (MWCO: 3500Da; Spectrum Medical Industries, Inc., Los Angeles, CA, USA) for 36 h at 4°C against distilled water. Finally, the synthesized polymer was lyophilized and stored at -70°C.

Characterization of PMGT

^{1}H NMR spectra of MDM and PMGT in D_2O were recorded using an Advanced 600 spectrometer (Bruker, Germany). The absolute molecular weight of PMGT was measured by gel permeation chromatography coupled with multiangle laser light scattering (GPC-MALLS) using a Sodex OHpack SB-803 HQ (Phenomenex, Torrells, CA, USA) column (column temperature 25°C; flow rate 0.5 ml/min).

Statistical analysis

Statistical significances were determined using student's t-test or one-way ANOVA. All quantitative results were presented as mean ± standard deviation (SD).

Table T1: Characterization of PMGT

[a] Determined by ^{1}H NMR [b] Determined by GPC

Sample	MW of reactants [Da]		MDM : bPEI [mol/mol]	Composition of bPEI [a] [mol-%]	Composition of MDM [a] [mol-%]	Molecular weight [b] [Da]	Polydispersity index (PDI)	Yield
	MDM	bPEI						
PMGT	318.32	1200	1 : 1	63.3	36.7	8592	1.312	42%
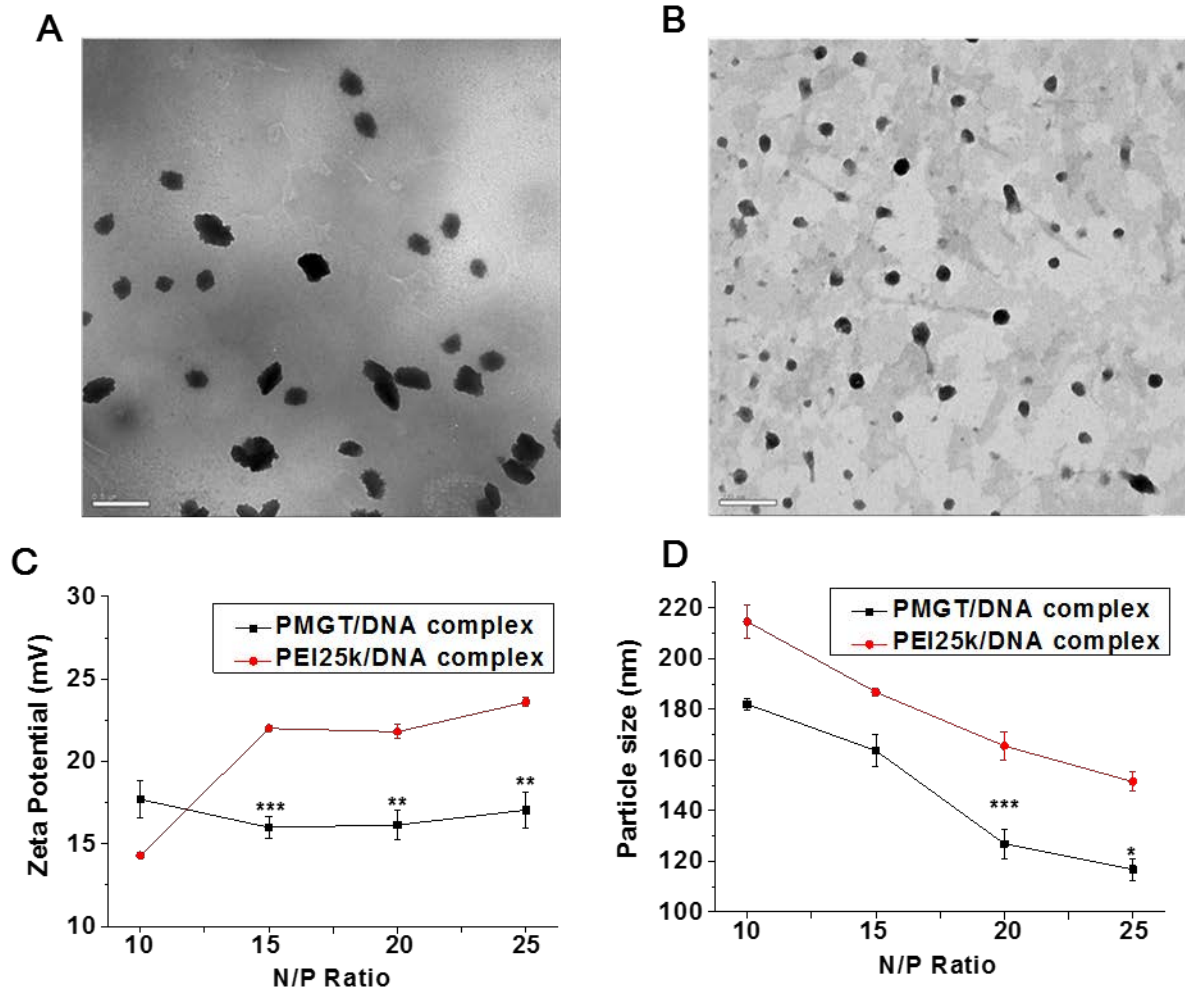
Fig. S1 The physicochemical characterization comparison of PMGT/DNA and PEI/DNA complexes. (A) EF-TEM images of PMGT/pGL3 and PEI25k/pGL3 complexes at an N/P ratio of 20. (D) PMGT/pGL3 particle size without serum and (E) PMGT/pGL3 zeta potential at different N/P ratios (n = 3, error bar represents SD) (*p < 0.05; **p < 0.01; ***p < 0.001, one-way ANOVA).
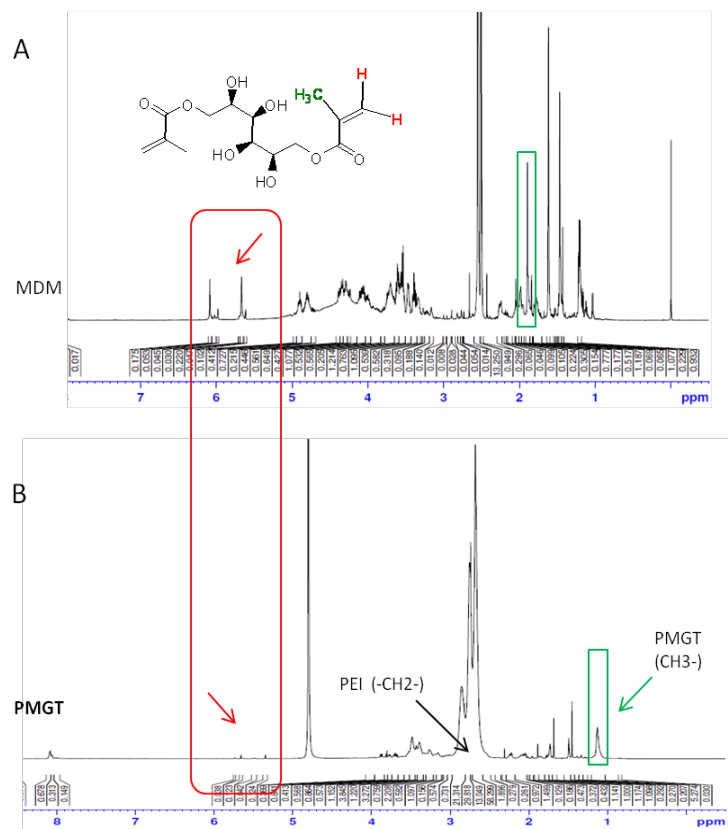
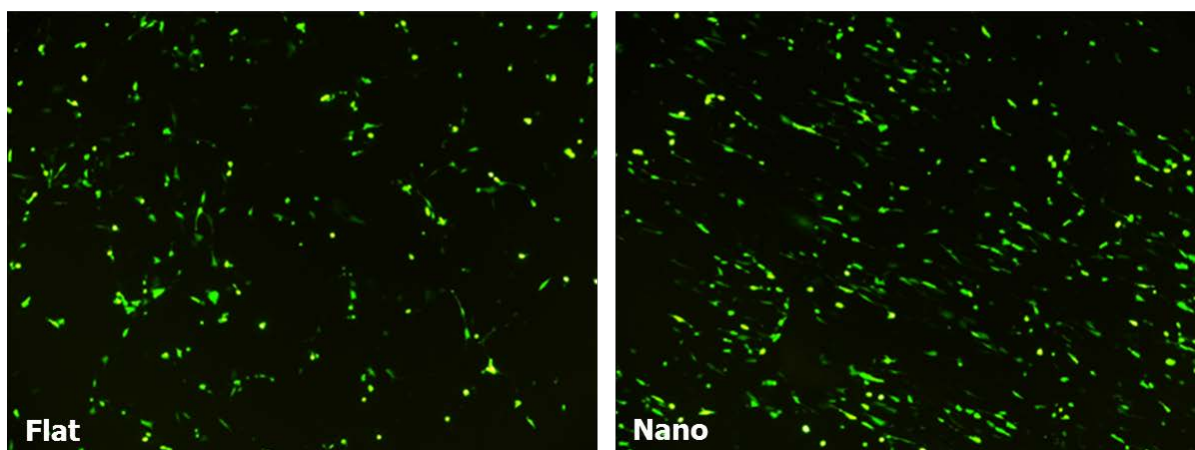**Fig. S2.** Characterization of PMGT copolymer: ^{1}H-NMR spectra of (A) MDM in DMSO and (B) PMGT in D_2O
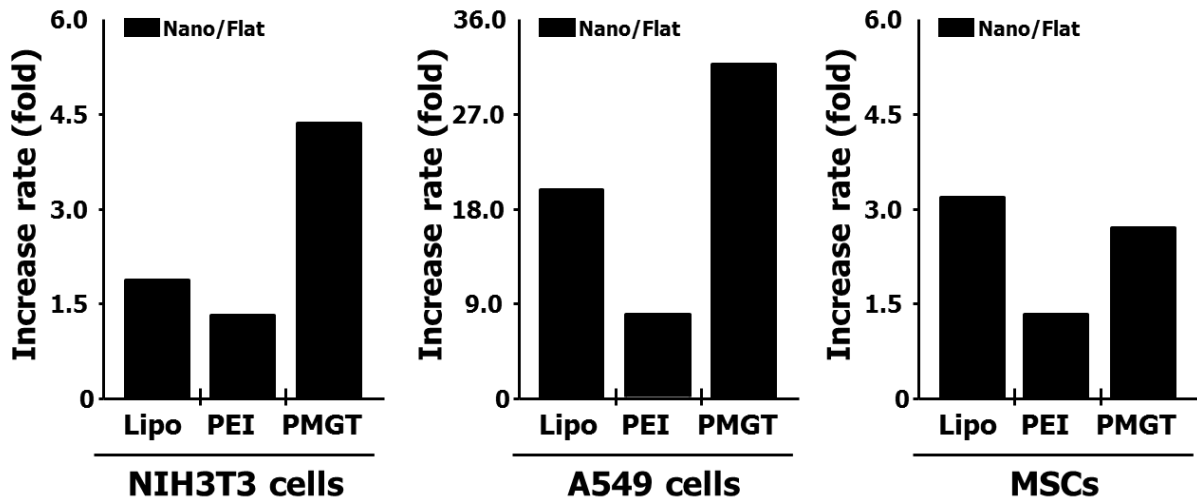
Fig. S3 Quantitative analysis of the data on gene transfection of vector/pGL3 complexes in various cells (Figs. 3 and 4). The "Nano/Flat" means the value of gene transfection on the nanopatterned matrix divided by that on the flat substrate.

PMGT

Fig. S4 Representative fluorescent images of PMGT/tGFP complexes in NIH3T3 fibroblasts cultured on substrates, showing the enhanced transfection on the nanopatterned matrix.
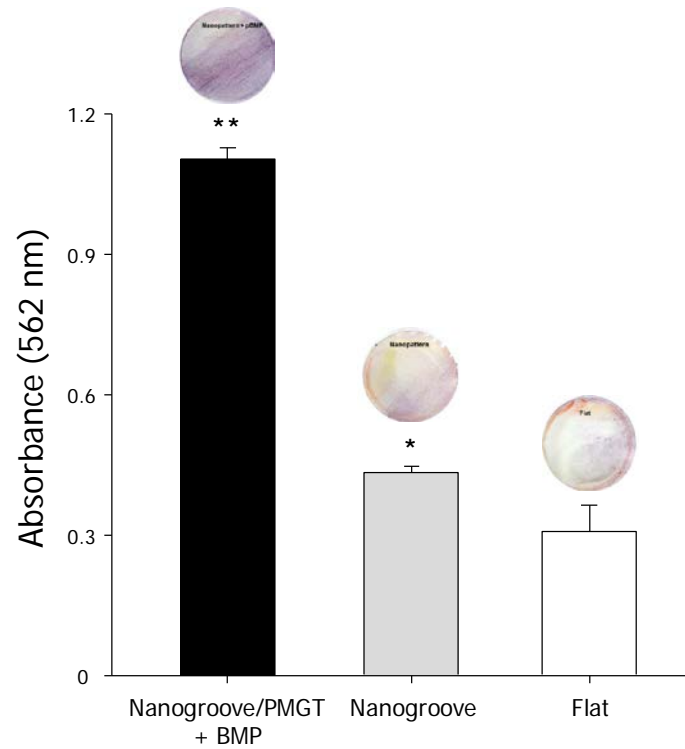
Fig. S5 Alizarin staining of MSCs cultured on the flat and nanopatterned surfaces (500 nm size), showing osteogenic differentiation after 3 weeks. Images showing highest alizarin staining in cells grown on nanopatterned matrix combined with PMGT/pBMP transfection. The quantification of the degree of mineralization as measured by Alizarin staining. Error bars represent the SD about the mean (n = 3 for each group). *p < 0.05; **p < 0.01.