Supporting Information

Apatite-binding nanoparticulate agonist of hedgehog signaling for bone repair

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Simulated Body Fluid (SBF). To prepare 5×SBF 1 (ion concentrations were 5 times compared to human plasma), 344.4 mg $CaCl_2$, 379.4 mg $MgCl_2$, 441.3 mg $NaHCO_3$ and 285.0 mg K_2HPO_4 were dissolved into 250 mL deionized water. After the pH was adjusted to 6.0, 88.8 mg Na_2SO_4, 278.1 mg KCl and 9.9 g NaCl were added. 5×SBF 1 was obtained after adjusted pH to 6.5 and sterilized using a filter containing 0.22 μm membrane. For 5×SBF 2 (Mg^{2+} and HCO_3^- free), 344.4 mg $CaCl_2$ and 285.0 mg K_2HPO_4 were dissolved into 250 mL deionized water. After the pH was adjusted to 6.0, 111.3 mg KCl and 10.4 g NaCl were added. 5×SBF 2 was obtained after adjusted pH to 6.8 and sterilized using a filter containing 0.22 μm membrane. Both 5×SBF 1 and 5×SBF 2 were stored at 4 □ for future use.

Western Blot Assay. BMSCs cells were seeded into Ap-PLGA and MNA-loaded Ap-PLGA scaffolds for 48 h. Cells were washed with ice-cold PBS 3 times and total cell lysates were gathered after addition of 100 μL RIPA lysis buffer at 4 °C. Protein extracts were isolated from cell lysates after centrifugation and quantified using BCA Protein Assay Kit (Thermo Fisher Scientific). An equal amount of protein (30 μg) from each sample was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membrane. Membranes were blocked at room temperature for 1 h in blocking buffer containing 5% nonfat dry milk to prevent nonspecific binding and then separately incubated with anti-Shh, anti-Gli1 and anti-GAPDH primary antibodies at 4 °C overnight. After washing with Tris-buffered saline containing 0.4% Tween-20 for 30 min and incubation with secondary antibodies (goat antirabbit IgG HPR) for 2 h, the membranes were visualized with ECL detection kit (Thermo Fisher Scientific) by a UV illuminator (Bio-Rad ChemiDoc XRS+).

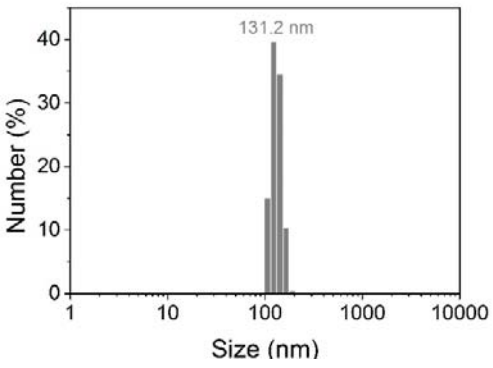

Figure S1. Size distribution of STs as measured using DLS. Zeta potential of STs was

-25.1 mV.

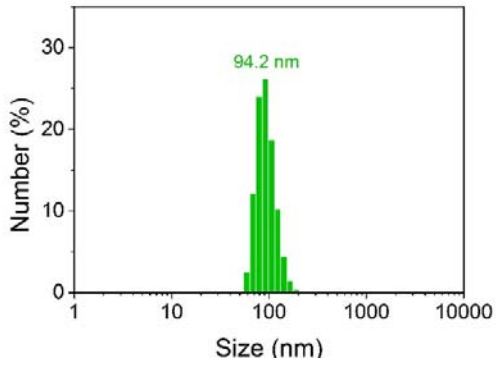

Figure S2. Size distribution of ASTs as measured using DLS. Zeta potential of ASTs was -24.1 mV.

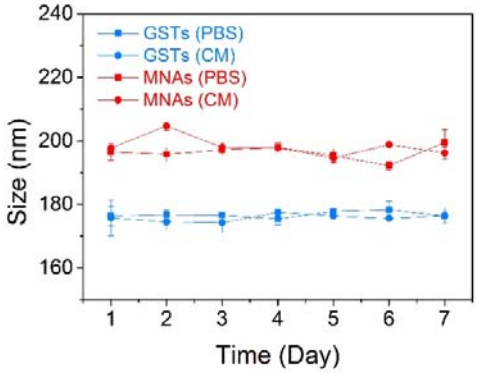

Figure S3. Size stability of GSTs and MNAs in culture medium (CM) containing 10% FBS and PBS over 7 days as measured using DLS.

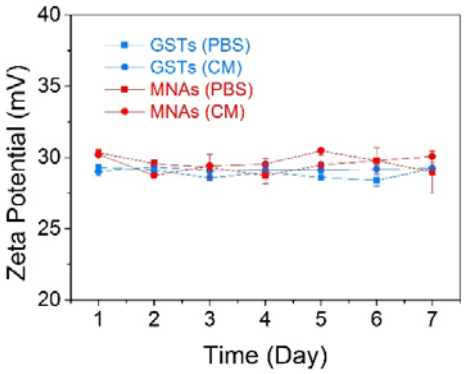

Figure S4. Zeta potential stability of GSTs and MNAs in culture medium (CM) containing 10% FBS and PBS over 7 days as measured using DLS.

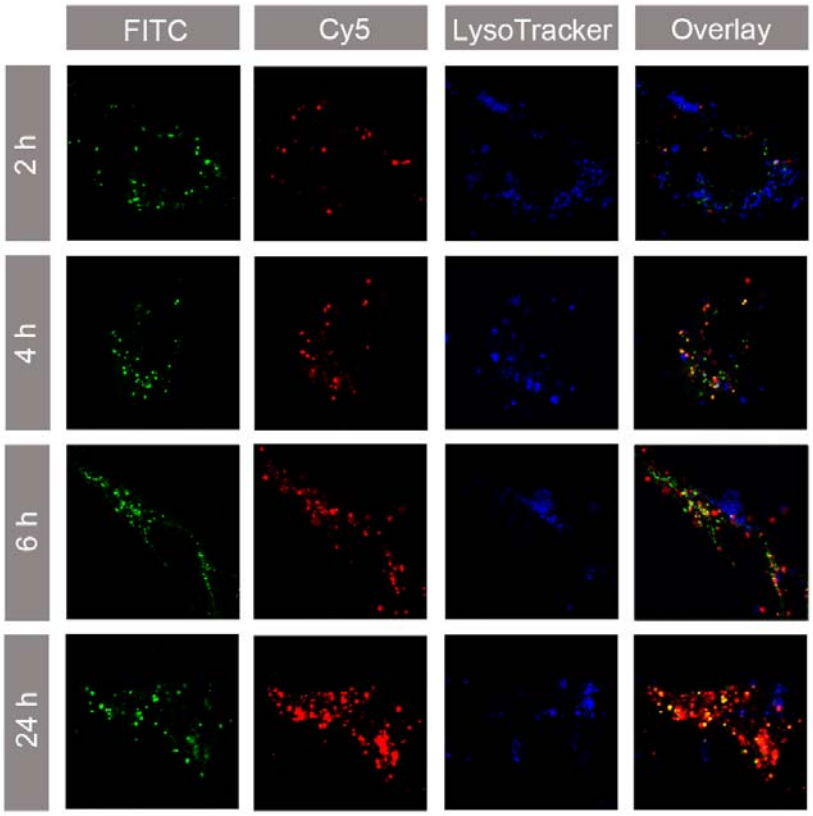

Figure S5. CLSM images for BMSCs after incubation with MNAs over 24 h, including FITC-labeled PEI channel (green), Cy5-labeled pDNA channel (red), Lysotracker-stained lysosome channel (blue) and overlay of previous images.

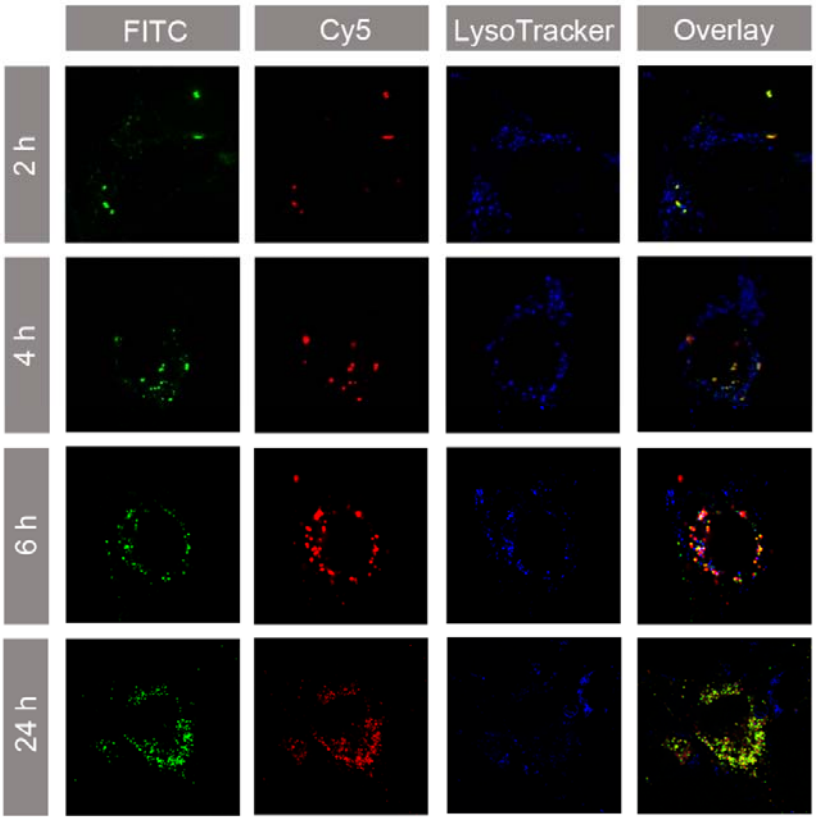

Figure S6. CLSM images for BMSCs after incubation with PEI/pDNA complex over 24 h, including FITC-labeled PEI channel (green), Cy5-labeled pDNA channel (red), Lysotracker-stained lysosome channel (blue) and overlay of previous images.

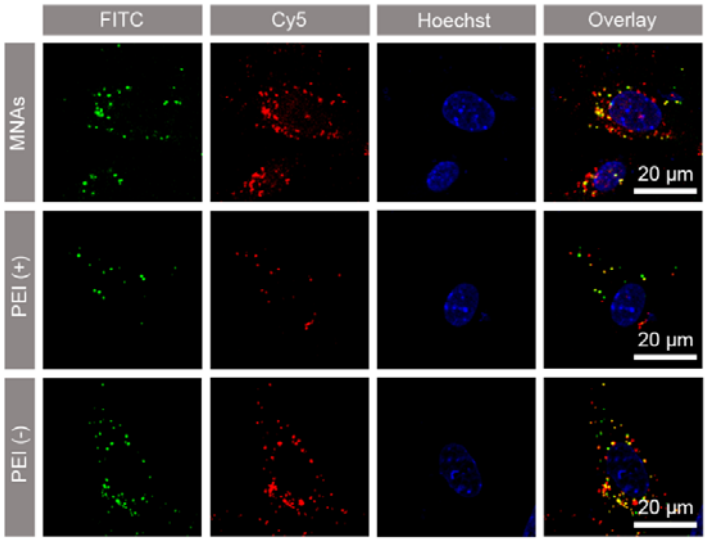

Figure S7. CLSM images for BMSCs after incubation with MNAs and PEI/pDNA complex for 24 h, including FITC-labeled PEI channel (green), Cy5-labeled pDNA

channel (red), Hoechst-stained nucleus channel (blue) and overlay of previous images.

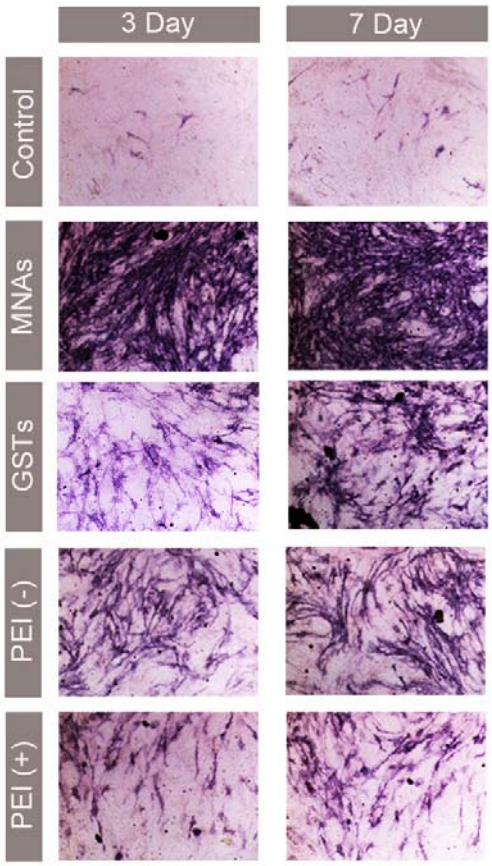

Figure S8. Expression of ALP in BMSCs incubated with 15 μg/mL STs, ASTs, GSTs and MNAs in culture medium for 2 days and treated with osteogenic medium for another 3 or 7 days.

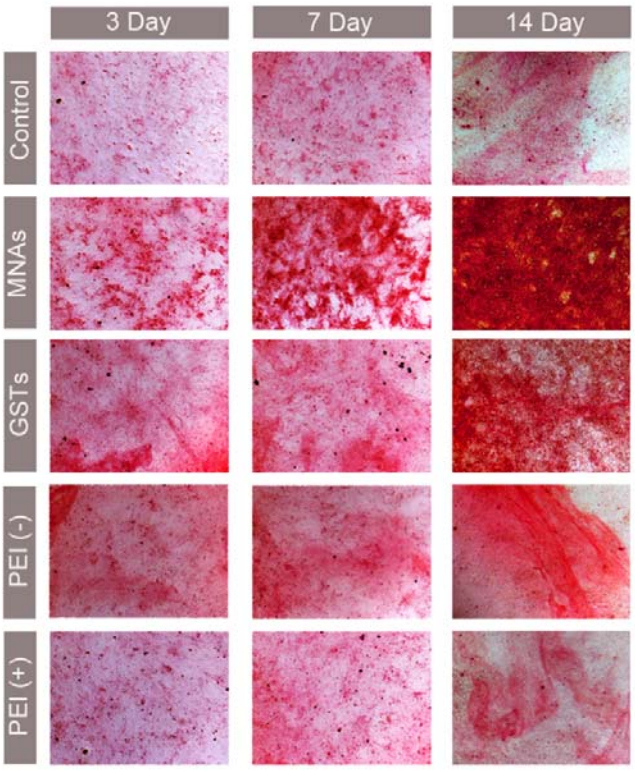

Figure S9. Mineralization in BMSCs after incubation of GSTs, MNAs, PEI in culture medium for 2 days and treatment with osteogenic medium for another 3, 7 and 14 days.

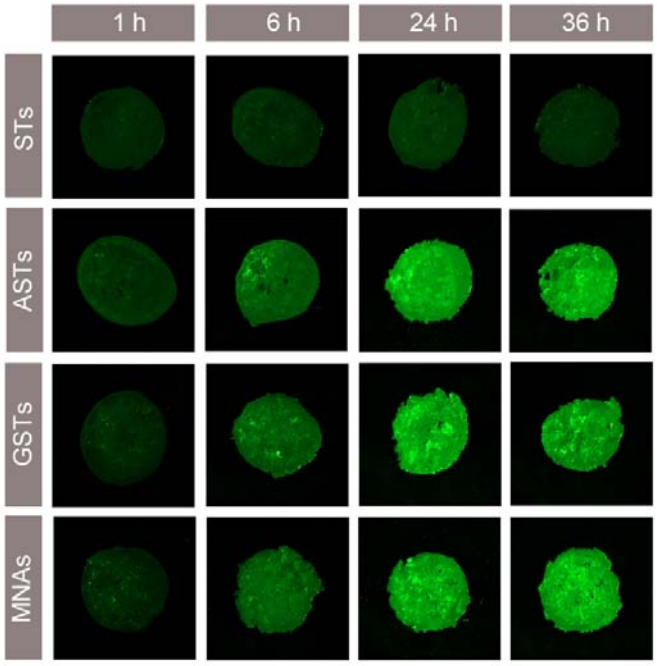

Figure S10. Fluorescent images of Ap-PLGA scaffolds after incubation of 100 μg/mL STs, ASTs, GSTs and MNAs over 24 h.

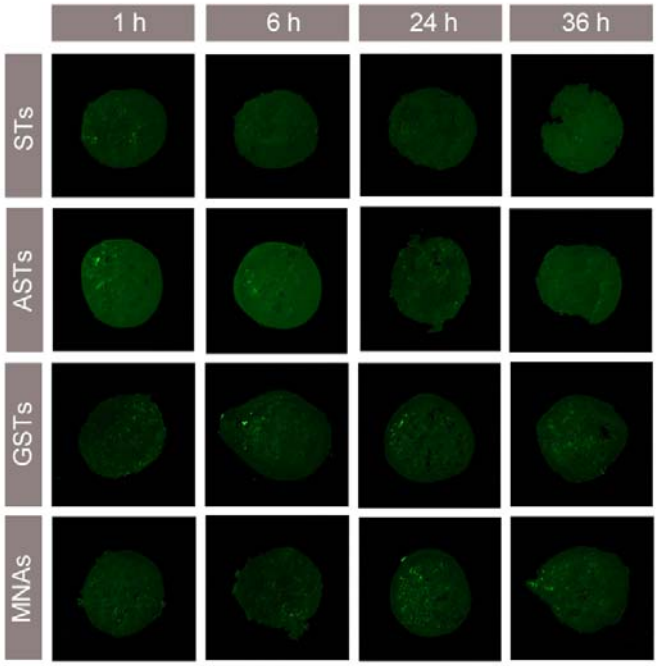

Figure S11. Fluorescent images of PLGA scaffolds after incubation of 100 μg/mL STs, ASTs, GSTs and MNAs over 24 h.

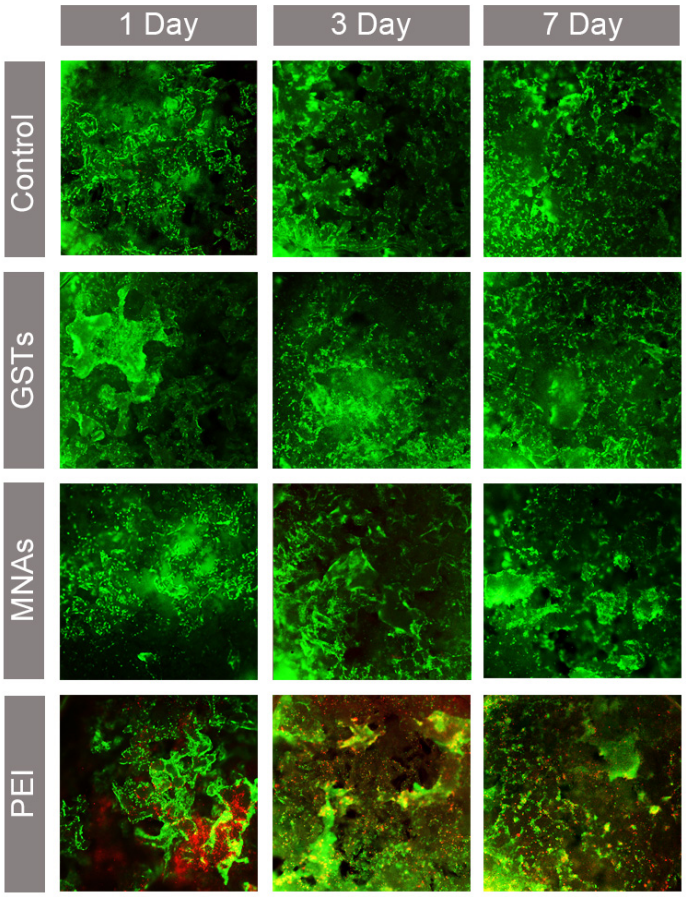

Figure S12. Live/Dead staining of Ap-PLGA, GST-bound Ap-PLGA, MNA-bound Ap-PLGA and PEI-bound Ap-PLGA scaffolds after seeding BMSCs and culturing for 1, 3 and 7 days.

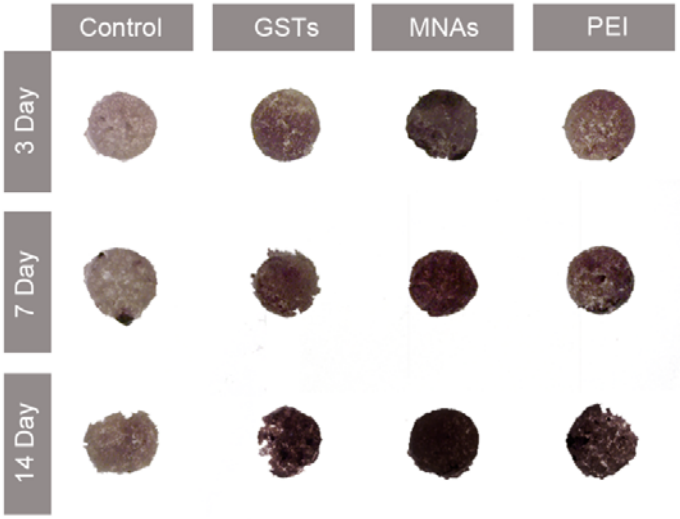

Figure S13. ALP expression of BMSCs on Ap-PLGA, GST-bound Ap-PLGA, MNA-

bound Ap-PLGA and PEI-bound Ap-PLGA scaffolds after incubation in osteogenic medium for 3, 7 and 14 days.

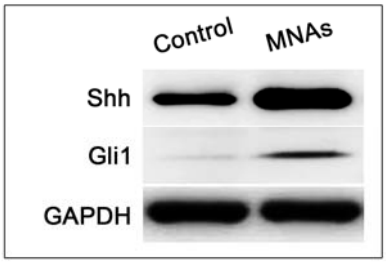

Figure S14. Protein expression of Shh and Gli1 of BBMSCs in Ap-PLGA scaffolds and MNA-loaded Ap-PLGA scaffolds on day 2.

Table S1. Sequences of primers for qPCR assay.

Primers	**Forward**	**Reverse**
GAPDH	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
ALP	GTTGCCAAGCTGGGAAGAACAC	CCCACCCCGCTATTCCAAAC
Runx2	CGGTCTCCTTCCAGGATGGT	GCTTCCGTCAGCGTCAACA
Col 1α1	AACCCGAGGTATGCTTGATCT	CCAGTTCTTCATTGCATTGC
OCN	GGGAGACAACAGGGAGGAAAC	CAGGCTTCCTGCCAGTACCT
Shh	GGATGAGGAAAACACGGGAGCA	TCATCCCAGCCCTCGGTCACT
Smo	TGCTACCTTACCAACCGTGGCT	ACGCTGTTCTGACTCTCGGCAT
Ptch	CAGGCTTCCTGCCAGTACCT	GACAATGATTCCAGCAGTCCAAG
Gli1	ACACATTACCAAGAAGCACCG	CAGCTGGTTTTCCCCTTTAAC