Particle
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Supporting Information

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Monodispersed #-Glycerophosphate-Decorated Bioactive Glass Nanoparticles Reinforce Osteogenic Differentiation of Adipose Stem Cells and Bone Regeneration In Vivo

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1. Experimental section

1.1 The characterization of BGN@GP

The chemical structures of BGN@GP nanoparticles were characterized by Fourier transformation infrared (FT-IR) spectroscopy (Nicolet 6700, Thermo Scientific Instrument). Briefly, KBr was used as a reference in the detection of FT-IR, subsequently, the nanoparticles were mixed with KBr and the mixture was pressed into a transparent slice for measurement. The spectra were obtained from 4000 to 400 cm^{-1} at a scan resolution of 4 cm^{-1}. The surface morphology was observed by transmitted electron microscope (TEM, H-8000, Hitachi). In brief, the nanoparticles were dispersed in ethanol; then observed the nanoparticles by TEM. Energy dispersive spectroscopy (EDS) was detected by field emission scanning electron microscope (FE-SEM, SU8010, Hitachi). We fixed BGN@GP at sample stage and observed. We detected the amount of β-glycerophosphate functionalized on the BGNs by thermogravimetric analysis (TGA/DSC3, Mettler Toledo). This instrument supplies a continuous measurement of sample weight as a function of time or temperature. BGN@GP weighing around 7 mg were placed in a pottery crucible and heated.

1.2 The cytotoxicity evaluation and the ions release

The cytotoxicity was measured by using an Alamar blue® assay (Invitrogen) In brief, cells were seeded on the 96-well plates with a density of 1500 cells/cm^2, and the BGN@GP nanoparticles were added into the medium when the cells attached to the plates. Subsequently, the cells were incubated in the medium containing 10% (v/v) Alamar Blue dye at 37 °C with 5% CO_2 for 4 h. 75 μL medium of each example was scan by a Spectra Max fluorescence microplate reader (Molecular Devices).Live/dead fluorescence staining were performed to observe the situation of

the cells. Briefly, the cells were seeded on 96-well plates, and the BGN@GP was added into the medium after 8 hours' cultivation. The cells were slightly washed with phosphate buffer saline (PBS) two times, ethidium homodimer-1 (0.5 μM) and calcein AM (0.25 μM) (Life technologies) were added to the serum-free medium; then the cells were incubated with the work medium at the cell culture condition for 45 minutes. Finally, cells were observed by an inverted fluorescence microscope (IX53, Olympus). Red color represented that the cell was dead, green color showed that the cell was alive.

For the release of ions, both GP-BGN and BGN were dispersed in PBS with a concentration of 2 mg mL^{-1}. The nanoparticles-contained solutions were incubated with growth medium (DMEM+15%FBS) and osteogenic differentiation medium at room temperature. Medium was refreshed after dispersed for 1 days, and 5 days. The released ions amount in the medium was detected by ICP-MS (7500CE, Agilent) to further study the influence of GP functionalized BGN. The release of GP was determined by the potentiometric titration method. Briefly, the BGN@GP was placed in a dialysis tube (MWCO 3500). After dialysis for 24 h, the content of GP in the dialysis solution was determined by the volume of the titrant. The titrant is 0.05 mol/L sulfuric acid solution.

1.3 The H.E and Masson staining

The histochemical staining was employed to analyze the repair and regeneration of bone tissue. Rats were euthanized with two times of the normal dose of 10% chloral hydrate at 8w and 12w; regenerated tissues were harvested and fixed with 10% formalin. The fixed tissue was soaked in the decalcified solution and placed on a shaker at room temperature. The decalcified solution was changed every 3-4 days. The decalcified process needed 2-3 weeks. After decalcification, the fixed

tissues were embedded in paraffin and sliced at 3-5 mm. The sections were stained following the procedure of Hematoxylin-Eosin staining kit (Beyotime). In brief, sample slices were removed paraffin by soaking in dimethylbenzene for 5-10 min. And we used different concentration of ethanol to dehydrate and washed the slices with distilled water. Then the slices were incubated with hematoxylin staining solution for 5-10 min. Subsequently, the slices were rinsed by tap water and distilled water. Next, the slices were embedded in eosin staining solution for 0.5-20 min. Finally, the slices were dehydrated and sealed. What's more, the process of Masson staining was similar with the procedures of H.E. staining. We treated the slices as previous description, soaking Masson staining solution for 5-10 min, then rinse, dehydration and seal. Then we observed the slices with optical microscope.

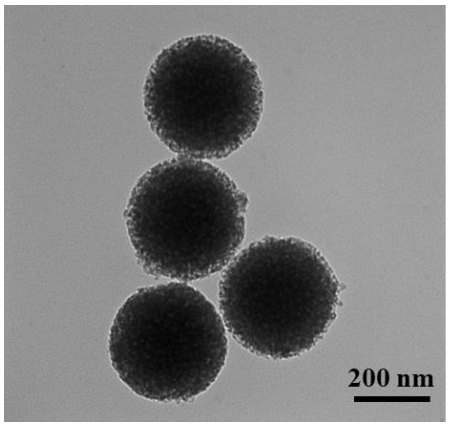

Figure S1. Morphology characterization TEM images of BGN (scale bar: 200 nm).

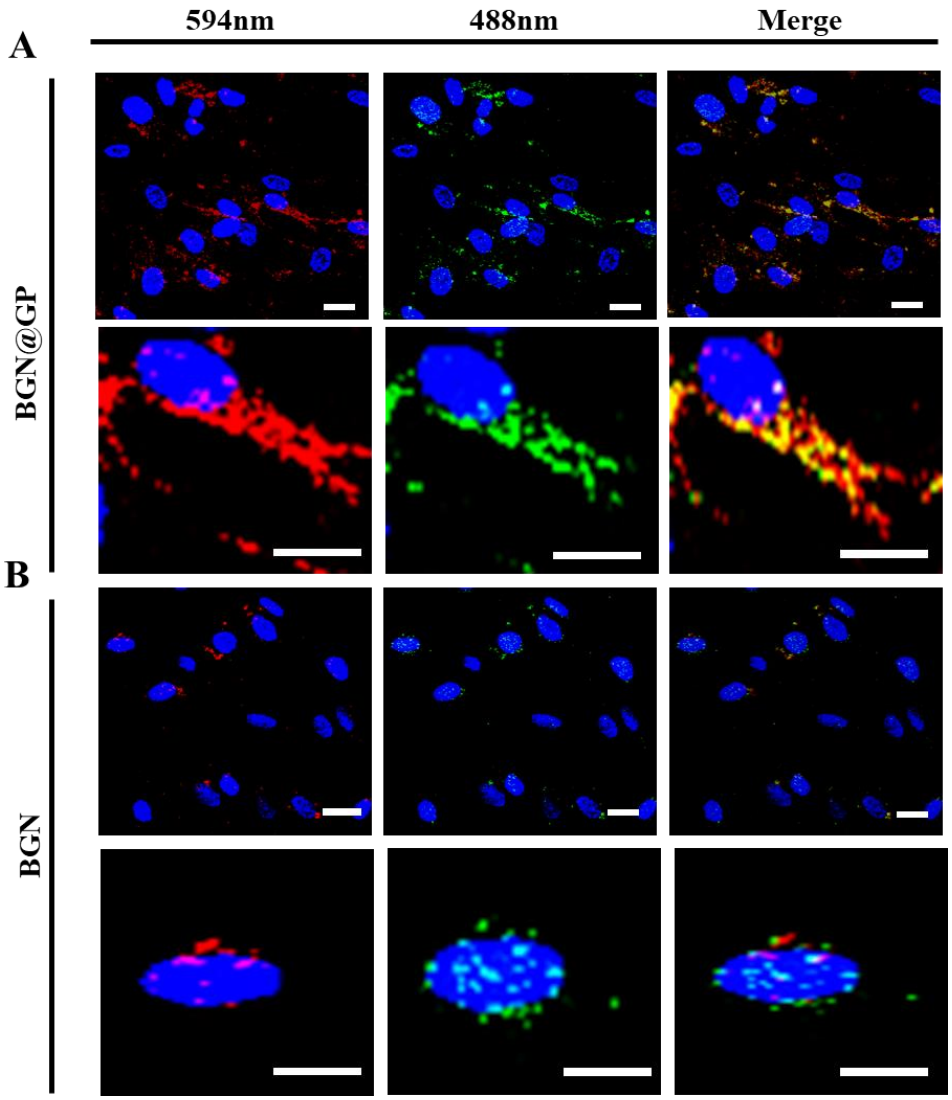

Figure S2. Confocal images of live ADMSCs cells after stained by BGN@GPs and BGNs, the cell nuclei were stained blue. Red and green represent the materials. The concentration of BGN and BGN@GP was 100 μg/mL Scale bar=20 μm.

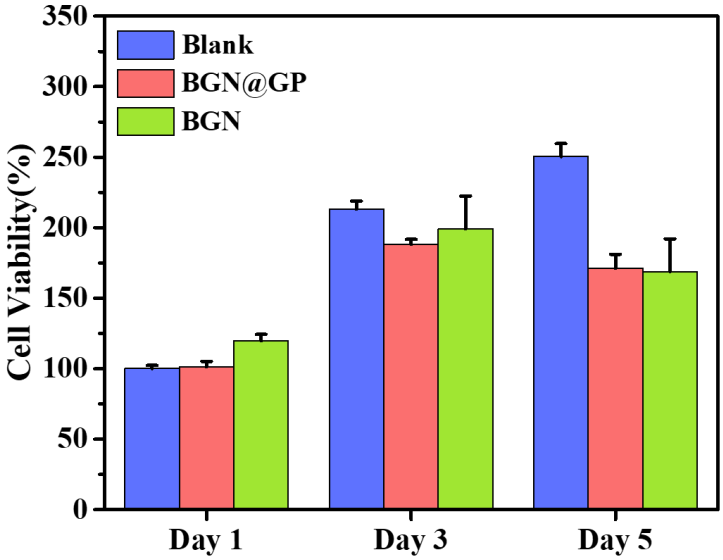

Figure S3. Cell viability evaluation of BGN@GP and BGN, using the concentration of 0 μg/mL as blank control. The concentration of BGN@GP and BGN was 60 μg/mL. ADMSCs viability and proliferation after culture for 1, 3, 5 days. *P<0.05 and **P<0.01.

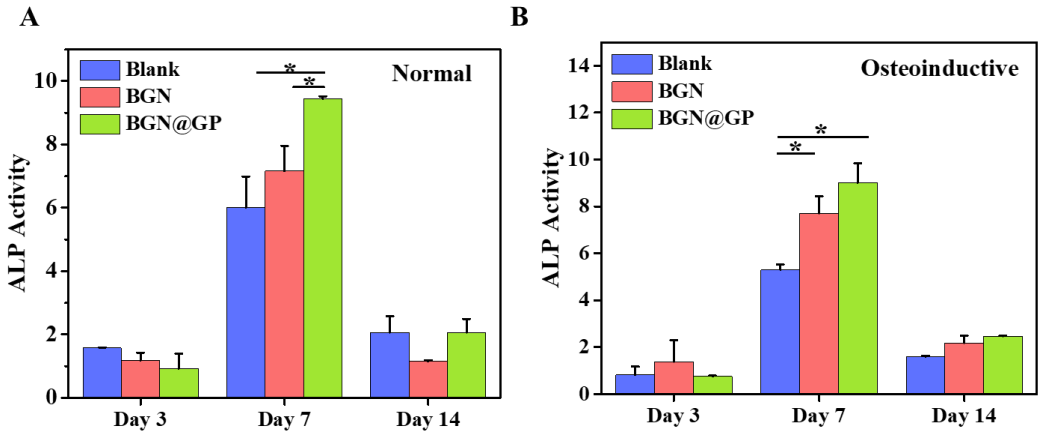

Figure S4. In normal and osteoinductive medium, the addition of BGN@GPs and BGNs upregulate ALP activity of ADMSCs; the ALP activity peak was obtained at day 7. The concentration of BGN@GP and BGN was 20 μg/mL. *P<0.05 and **P<0.01.

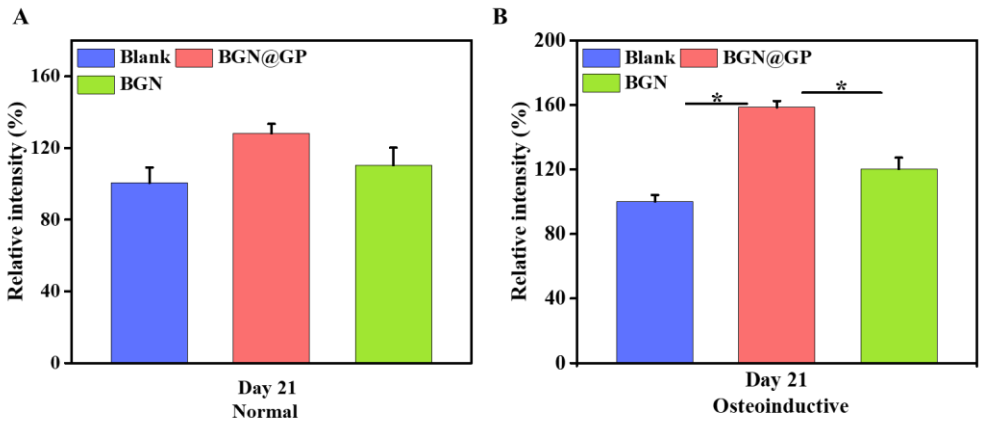

Figure S5. Alizarin red staining (ARS) assay at 21 days. (A-B) The images of ARS were gray level analyzed by image J. The concentration of BGN and BGN@GP was 80 μg/mL. *P<0.05 and **P<0.01.

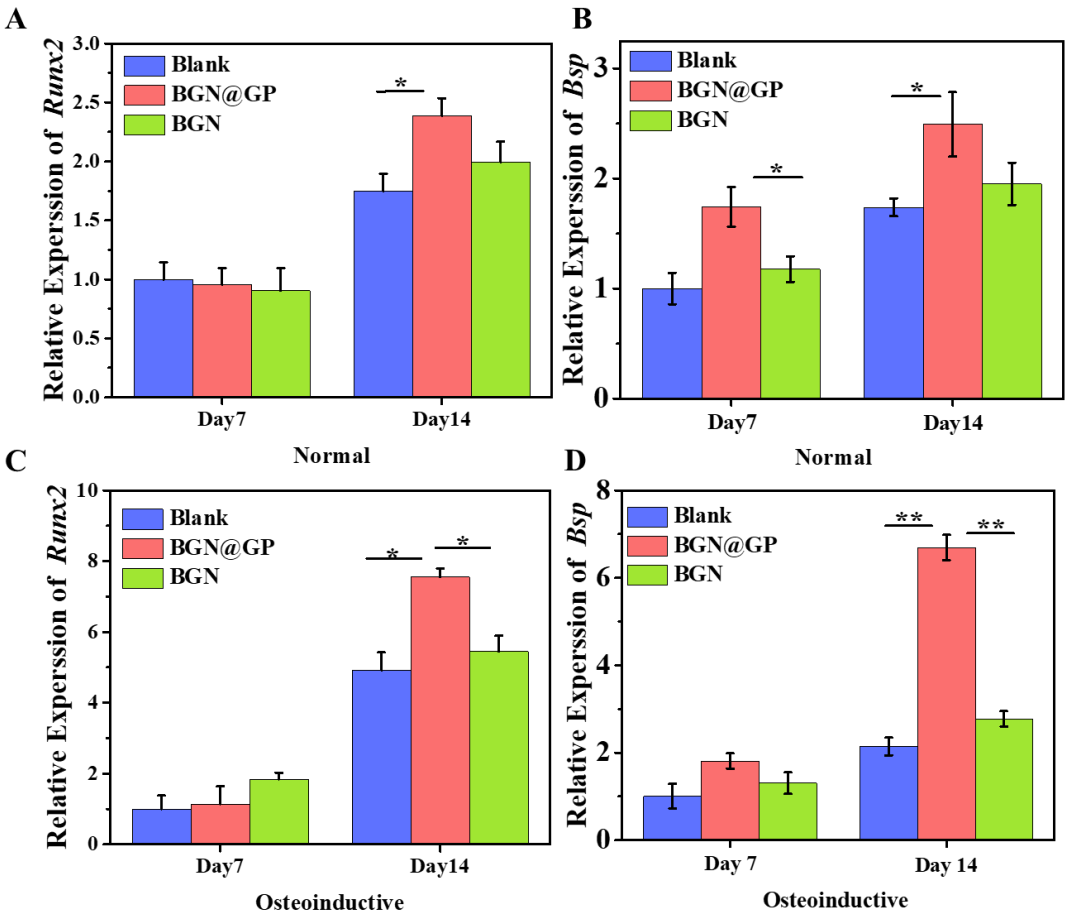

Figure S6. Relative expression of bone specific marker genes, namely *Runx2* and *Bsp*, by ADMSCs cultured on different medium with 80 μg/mL BGN@GP and BGN, during 7 and 14 days. The expression of these genes was normalized against the housekeeping gene *Gapdh* and calculated by the ΔΔCT method. (A-B) The relative expression of *Runx2* and *Bsp* with normal medium. (C-D) The relative expression of *Runx2* and *Bsp* with osteoinductive medium. *P<0.05 and **P<0.01.

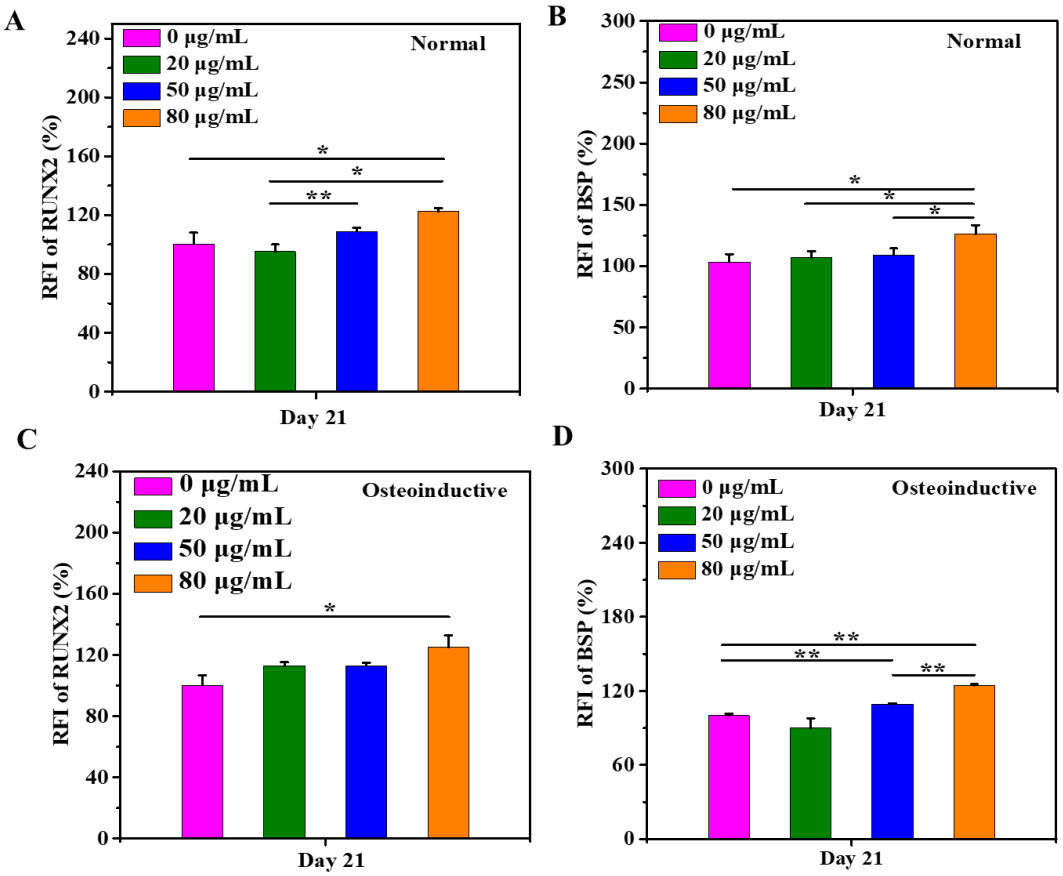

Figure S7. Immunofluorescent staining of bone specific genes RUNX2 and BSP at day 21 of osteogenic differentiation. The relative fluorescent intensity (RFI) of the immunofluorescent staining BSP was gray level analyzed. (A-B) The relative fluorescent intensity of RUNX2 and BSP immunofluorescent images with normal medium. (B) The relative fluorescent intensity of RUNX2 and BSP immunofluorescent images with normal medium. *P<0.05 and **P<0.01.

Table S1. Real-time quantitive PCR primers

Genes	Forward primer (5’-3’)	Reverse primer (5’-3’)
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Runx2	TCTTCCCAAAGCCAGAGCG	TGCCATTCGAGGTGGTCG
Bsp	ATGGCCTGTGCTTTCTCGAT	GTCACTGCTCGGAACTGGAA