

Supporting Information

Scaffold-Free Spheroids with Two-Dimensional Heteronano-Layers (2DHNL) Enabling Stem Cell and Osteogenic Factor Codelivery for Bone Repair

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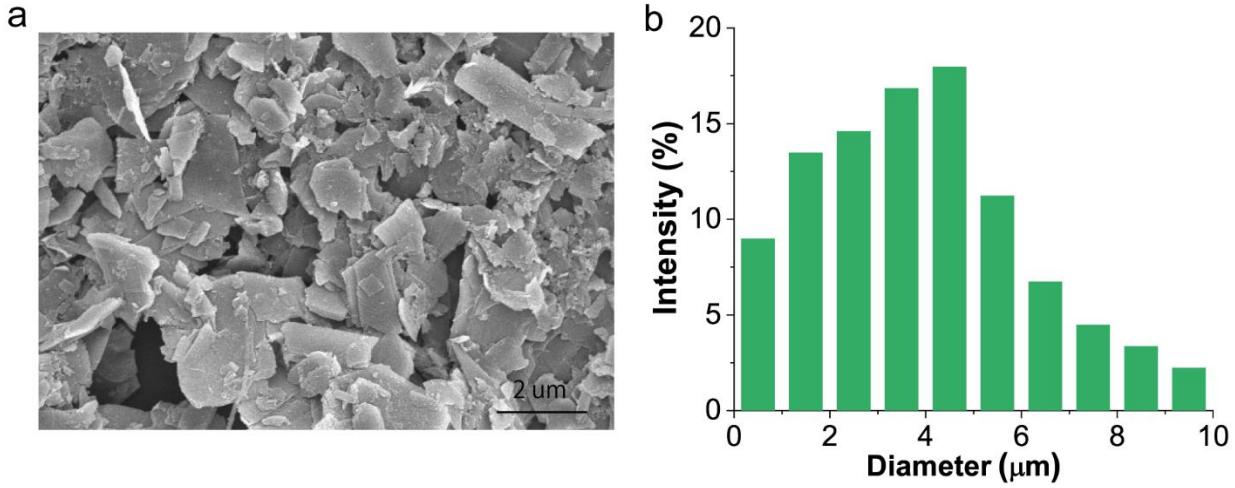


Fig. S1 The a) SEM and b) lateral size distribution of 2D BP nanosheets.

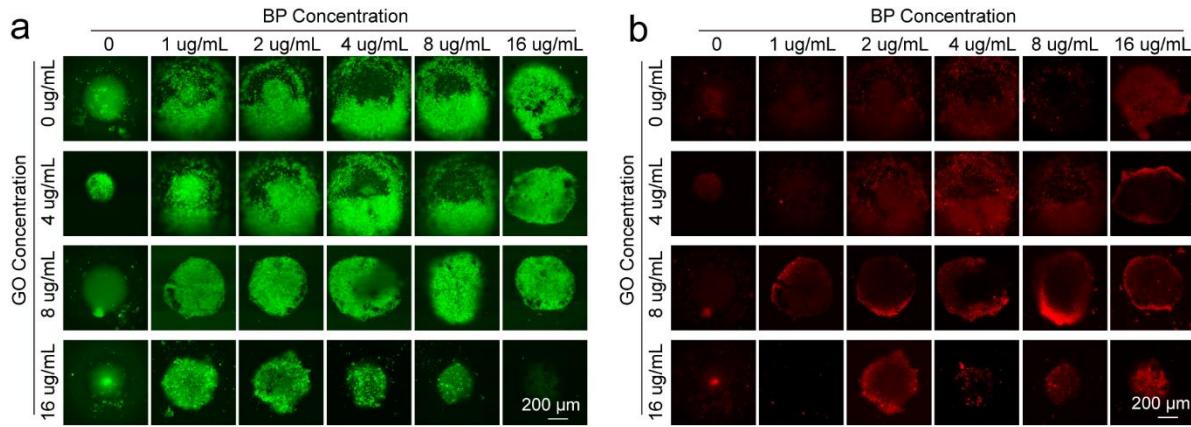


Fig. S2 Stem cell spheroids with a) live and b) dead staining after incorporation of various concentrations of GO and BP nanosheets after 3 days of culture.

Table S1. Rat-specific primers for PCR analysis.

Gene	Forward (5'->3')	Reverse (5'->3')
OPN	5'-GACGGCCGAGGTGATAGCTT-3'	5'-CATGGCTGGTCTTCCCCTGC-3'
OCN	5'-AAAGCCCAGCGACTCT-3'	5'-CTAACCGGTGGTGCCATAGAT-3'
Runx2	5'- GCTTCTCCAACCCACGAATG-3'	5'-GAAC TGATAGGACGCTGACGA-3'
BMP-2	5'-ATTGTGGCTCCCCGG-3'	5'-TCAGCCAGAGGAAAAGGGC-3'
OSX	5'-GATGGCGTCCTCTGTGCTT-3'	5'-TATGGCTTCTTGCTGCCCTCC-3'
GAPDH	5'-TGTGTCCGTGTTGGATCTGA-3'	5'-TTGCTGTTGAAGTCGCAGGAG-3'

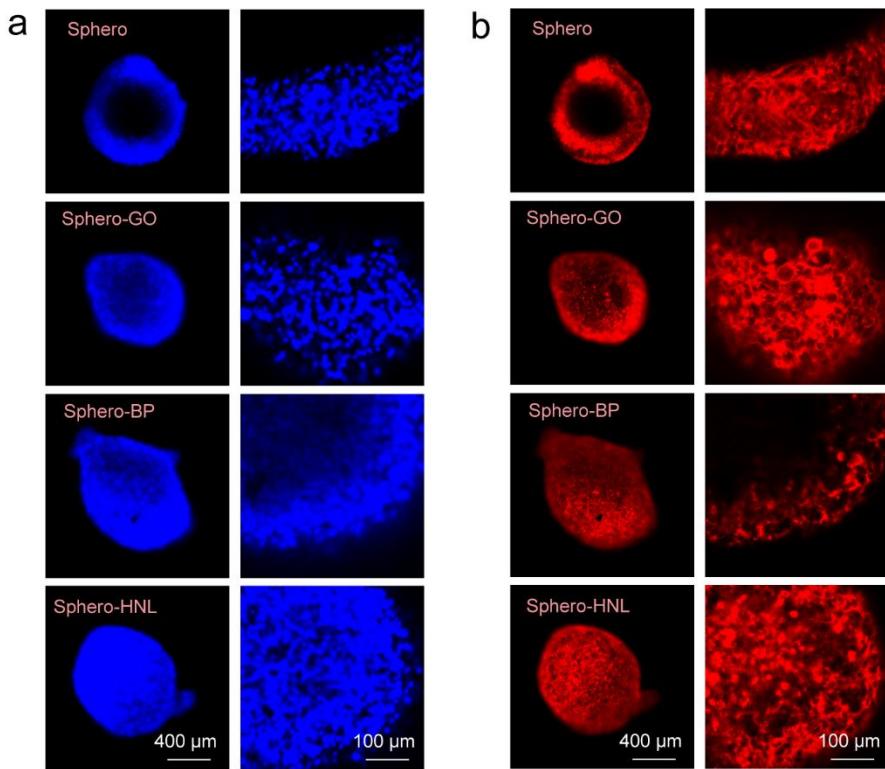


Fig. S3 Immunofluorescent images of a) nuclei stained by DAPI and b) filaments stained by rhodamine-phalloidin of MSC spheroids after 7 days of culture.

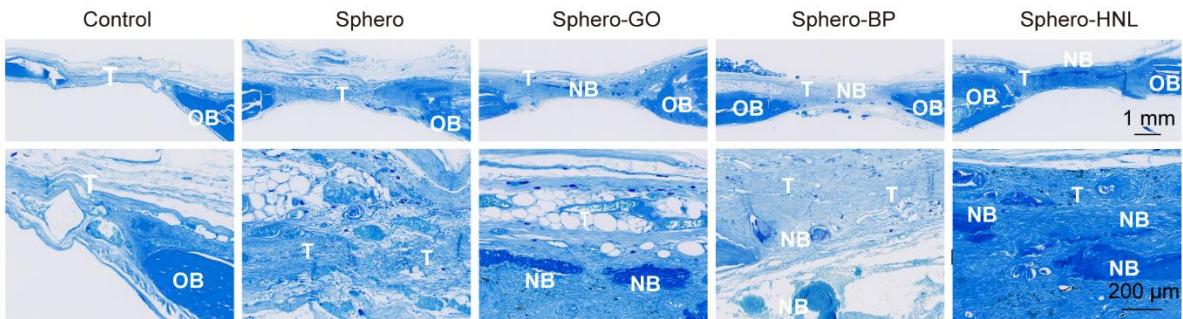


Fig. S4 Toluidine blue staining of tissue samples from rat cranial defects without (Control) or with stem cell spheroids after 8 weeks of implantation in the rat calvarial defect model. NB: New Bone; OB: Original Bone; T: Tissue.

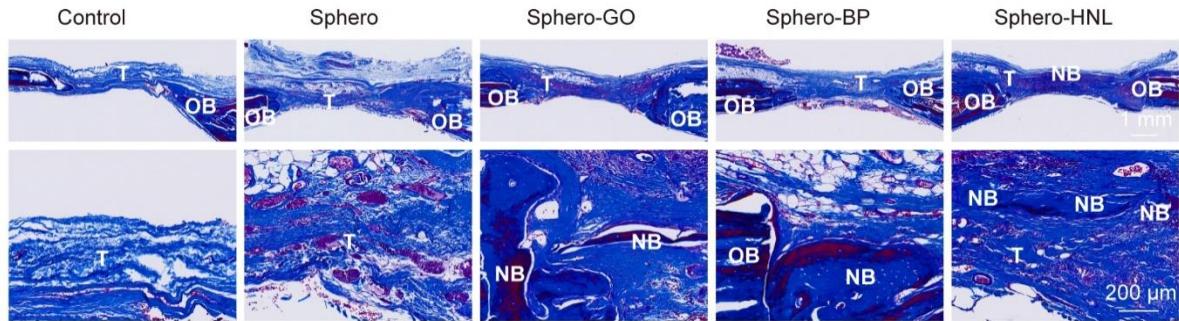


Fig. S5 Masson's trichrome staining of tissue samples from rat cranial defects without (Control) or with stem cell spheroids after 8 weeks of implantation in the rat calvarial defect model. NB: New Bone; OB: Original Bone; T: Tissue.

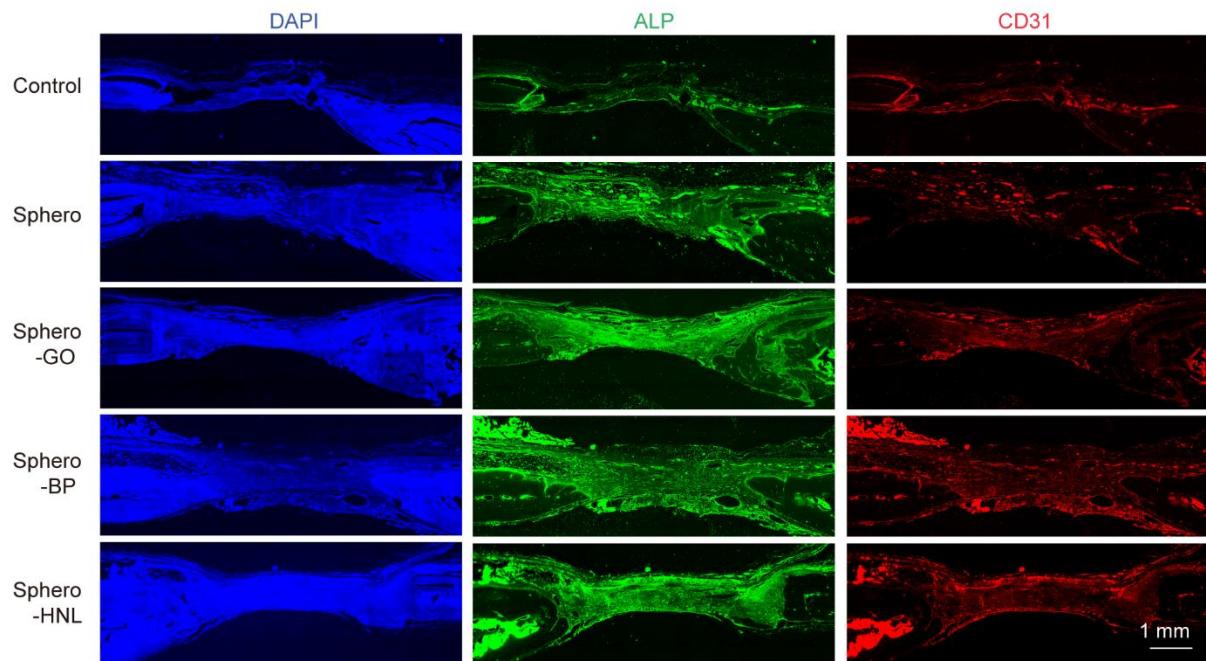


Fig. S6 Immunofluorescent imaging of nuclei (blue), osteogenic ALP protein (green), and vascular marker CD31 (red) in stem cell spheroids after 8 weeks of implantation in the rat calvarial defect model.