Recombinant human bone morphogenetic protein-2–engineered piezoplatform synergistically promotes bone regeneration through bone morphogenetic protein receptor activation

Supplementary file

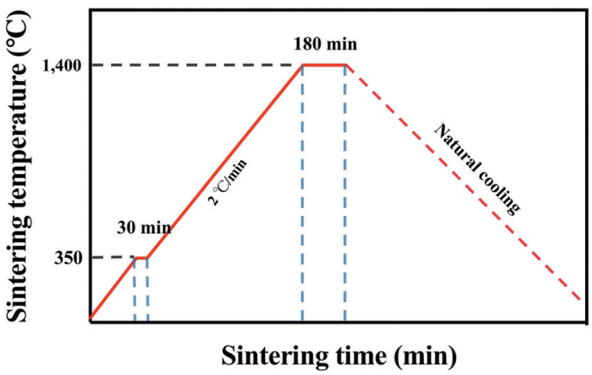

Figure S1. Sintering temperature program for barium titanate/tricalcium phosphate scaffolds

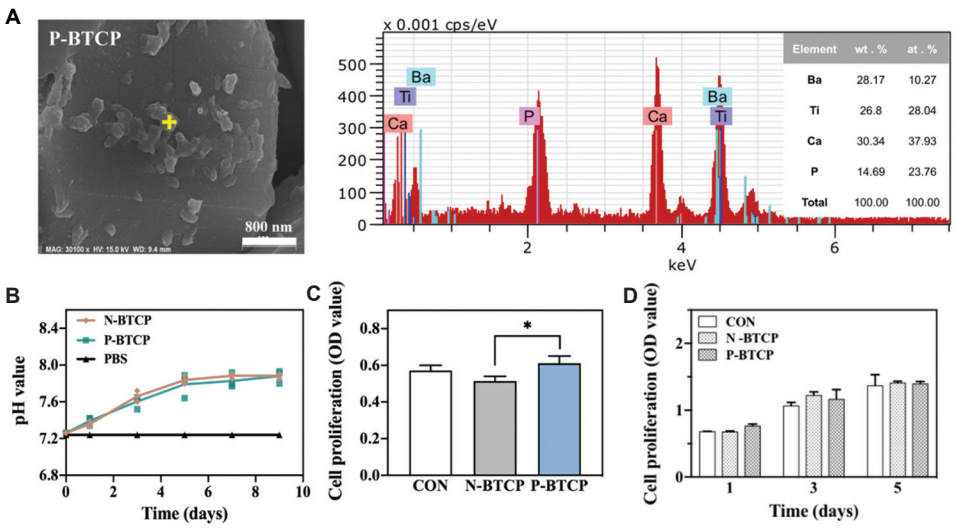

Figure S2. Characterization of scaffold and biocompatibility assessment. (A) Energy dispersive spectroscopy analysis (scale bar: 800 nm; magnification: 30,000×) and elemental composition of P-BTCP, showing newly formed mineralized grains after seven days of incubation. (B) pH values of N-BTCP and P-BTCP in PBS (pH 7.2) over 1–9 days. (C) BMSC proliferation on N-BTCP and P-BTCP scaffolds after three days of culture, measured by cell counting kit-8 assay. (D) Cytotoxicity of N-BTCP and P-BTCP extract media against BMSCs was evaluated by the cell counting kit-8 assay. *$p<0.05$.

Abbreviations: BMSC: Bone marrow stromal cell; CON: Control; N-BTCP: Non-piezoelectric barium titanate/tricalcium phosphate; OD: Optical density; P-BTCP: Piezoelectric barium titanate/tricalcium phosphate; PBS: Phosphate-buffered saline.

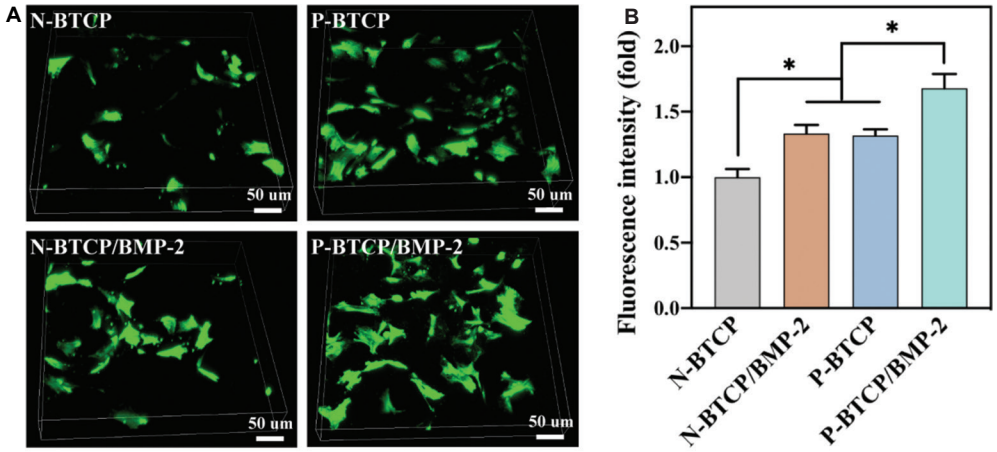

Figure S3. BMSC infiltration into BTCP scaffolds. (A) Confocal laser-scanning microscopy images (scale bar: 50 μm; magnification: 600×) and (B) quantitative analysis of fluorescence intensity of BMSCs infiltrating into N-BTCP, N-BTCP/BMP-2, P-BTCP, and P-BTCP/BMP-2 scaffolds after 24 h of culture. *p<0.05.
Abbreviations: BMP-2: Bone morphogenetic protein-2; BMSC: Bone marrow stromal cell; N-BTCP: Non-piezoelectric barium titanate/tricalcium phosphate; P-BTCP: Piezoelectric barium titanate/tricalcium phosphate.

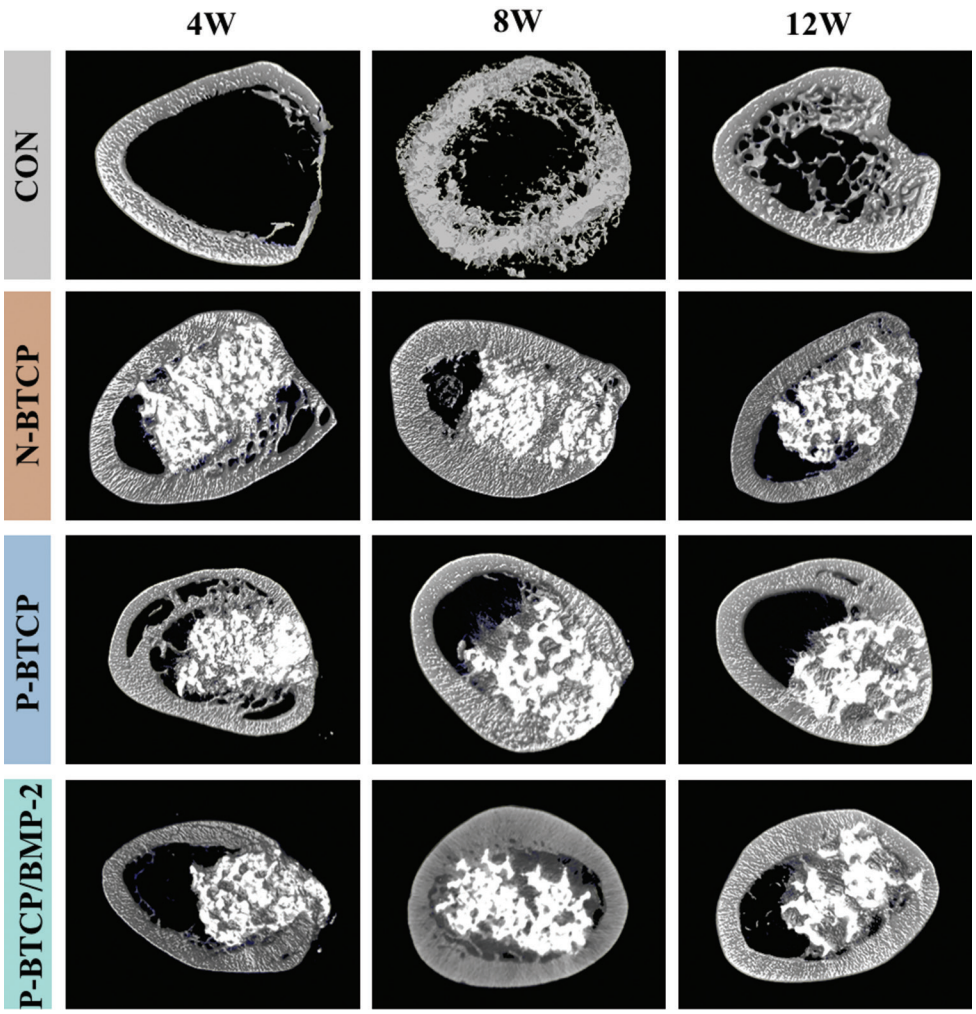

Figure S4. Micro-computed tomography images of new bone formation after implantation with N-BTCP, P-BTCP, and P-BTCP/BMP-2 scaffolds at 4, 8, and 12 weeks.
Abbreviations: BMP-2: Bone morphogenetic protein-2; CON: Control; N-BTCP: Non-piezoelectric barium titanate/tricalcium phosphate; P-BTCP: Piezoelectric barium titanate/tricalcium phosphate.

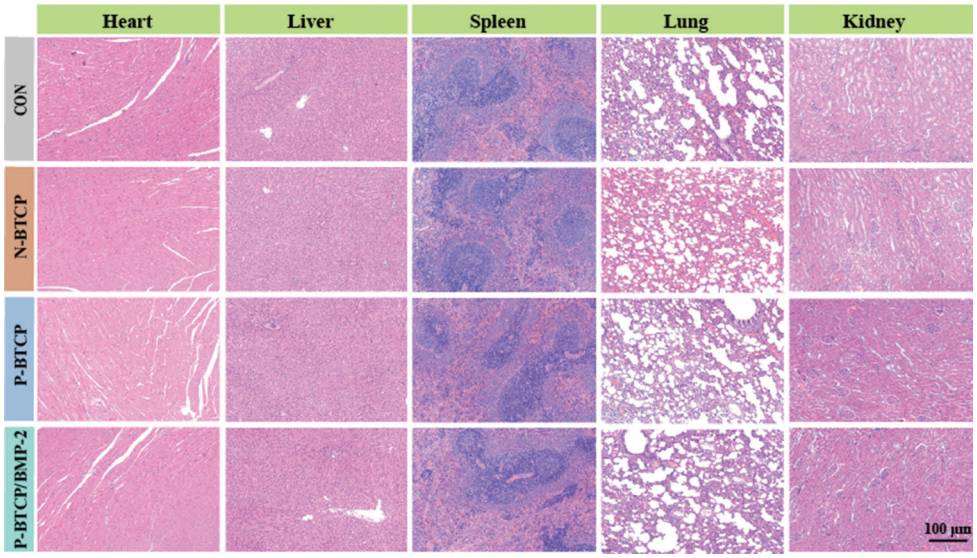

Figure S5. Hematoxylin–eosin histological analysis of major organs (heart, liver, spleen, lung, and kidneys) from Sprague–Dawley rats 12 weeks post-implantation with N-BTCP, P-BTCP, and P-BTCP/BMP-2 scaffolds (scale bar: 100 μm; magnification: 10×).
Abbreviations: BMP-2: Bone morphogenetic protein-2; CON: Control; N-BTCP: Non-piezoelectric barium titanate/tricalcium phosphate; P-BTCP: Piezoelectric barium titanate/tricalcium phosphate.

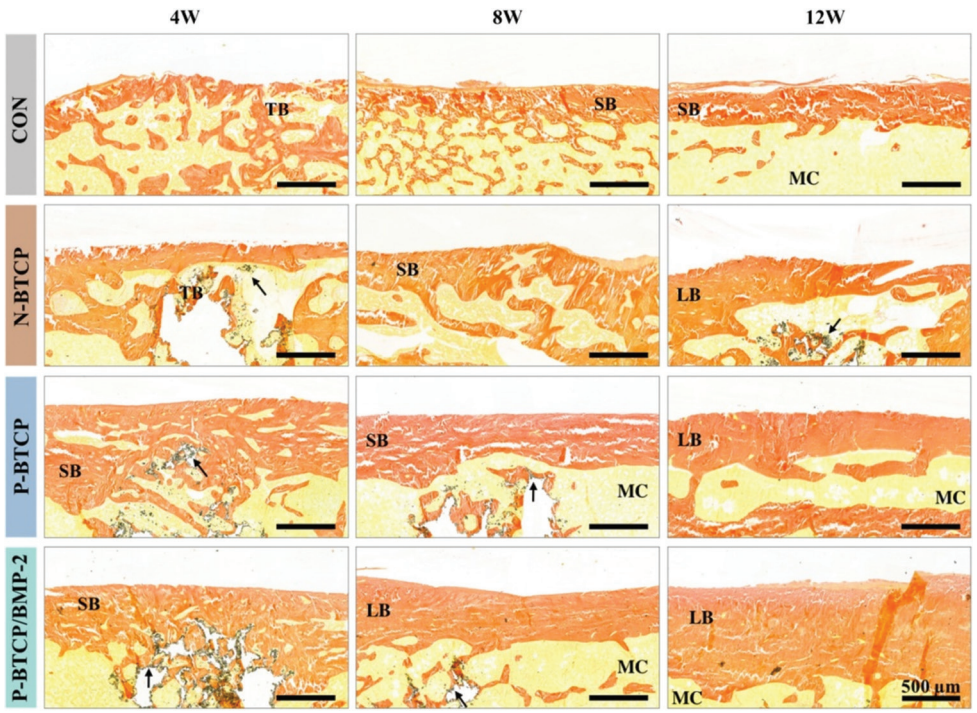

Figure S6. Van Gieson staining of undecalcified sections at 4, 8, and 12 weeks post- implantation, with black arrows indicating residual scaffolds (scale bar: 500 μm; magnification: 20×).
Abbreviations: BMP-2: Bone morphogenetic protein-2; CON: Control; LB: Lamellar bone; MC: Medullary cavity; N-BTCP: Non-piezoelectric barium titanate/tricalcium phosphate; P-BTCP: Piezoelectric barium titanate/tricalcium phosphate; SB: Spongy bone; TB: Trabecular bone.

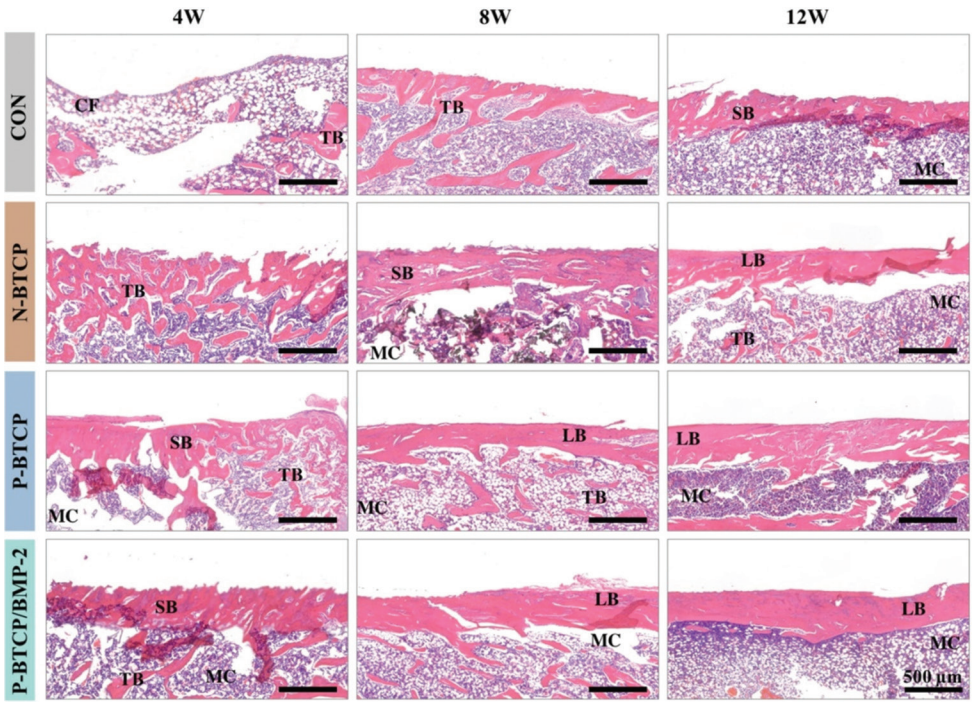

Figure S7. Hematoxylin–eosin staining of bone sections at 4, 8, and 12 weeks post-implantation (scale bar: 500 μm; magnification: 20×). Abbreviations: BMP-2: Bone morphogenetic protein-2; CF: Collagen fiber; CON: Control; LB: Lamellar bone; MC: Medullary cavity; N-BTCP: Non-piezoelectric barium titanate/tricalcium phosphate; P-BTCP: Piezoelectric barium titanate/tricalcium phosphate; SB: Spongy bone; TB: Trabecular bone.

Table S1. Primer sequences used in this study

Genes	Primer sequences
Bmpr1a	Forward: 5' - CAGTGATGAATGTCTTCGAGCC-3' Reverse: 5'-ACCCTTCCTCGGGTGAATTT-3'
Bmpr1b	Forward: 5'-GAGCAGTGACGAGTGCCTCAG-3' Reverse: 5'-CTTGGGCTAAGGATGCTTCTGT-3'
Bmpr2	Forward: 5'-GCCGGATTTCTCACCCGAAT-3' Reverse: 5'-GATTCTGGGAAGCAGCCGTA-3'
Smad1	Forward: 5'-CAGCAGCTACCCCAACTCTC-3' Reverse: 5'-CCGTCATGTTCGTCATGTTC-3'
Smad4	Forward: 5'-TCGATTCAAACCATCCAACA-3' Reverse: 5'-GCCCTGAAGCTATCTGCAAC-3'
Smad5	Forward: 5'-GAATGCCACGTTTCCTGATT-3' Reverse: 5'-AGGGGTATCAGCTGGGAGTT-3'
Smad8	Forward: 5'-GACAGCAGCATCTTTGTCCA-3' Reverse: 5'-CGTGCACATCTTCGTCAGTT-3'
Osx	Forward: 5'-GCCTACTTACCCGTCTGACTTT-3' Reverse: 5'-GCCCACTATTGCCAACTGC-3'
Run×2	Forward: 5'-TCCTGTAGATCCGAGCACCA-3' Reverse: 5'-CTGCTGCTGTTGTTGCTGTT-3'
Col1a1	Forward: 5'-CTGCCCAGAAGAATATGTATCACC-3' Reverse: 5'-GAAGCAAAGTTTCCTCCAAGACC-3'
Gapdh	Forward: 5'-CCTGCACCACCAACTGCTTA-3' Reverse: 5'-GGCCATCCACAGTCTTCTGAG-3'

Table S2. Macroporous parameters and mechanical strength of BTCP scaffold and reported TCP porous ceramic scaffolds

Scaffold	Pore size (μm)	Porosity (%)	Compressive strength (MPa)	Young's modulus (MPa)
β-TCP	350.0±150.0	-	0.009	-
TCP/MBG	350.0±150.0	-	0.4	-
BTCP	241.6±38.7	66.7±6.3	0.5±0.1	39.3±4.4

Abbreviations: β-TCP: β-tricalcium phosphate; BTCP: Barium titanate/tricalcium phosphate; MBG: Mesoporous bioactive glass.