

Supporting Information

S1. qPCR analysis of Ca²⁺ signal channels

qPCR experiments were performed to quantitatively detect the expression of mRNA related to L-type calcium ion (Ca²⁺) signaling pathways in bone tissue. The expression promoting effects of all scaffolds on various mRNA were shown in Fig. 1. Apparently, after co-culturing the cells with different scaffolds for 5 and 10 days, there were no significant differences in the levels of Cav1.1, Cav1.2, Cav1.3, and Cav1.4 in the absence of ultrasonic (US), indicating that each scaffolds had no apparent effect on the L-type Ca²⁺ signaling pathway.

Notably, cells co-cultured on PLLA/BTO and PLLA/BTO-GO for 5 days exhibited a 6.74-fold and 10.39-fold upregulation of Cav1.1 expression under US treatment (Fig. 1a), respectively. Meanwhile, other genes exhibited similar expression effects, which was completely different from the expression on PLLA under the same culture conditions.

On the 10 days, the expression of Cav1.1 in the PLLA/BTO and PLLA/BTO-GO group was significantly upregulated under US conditions. Especially, the expression level of Cav1.1 in PLLA/BTO-GO group maintained at 15.37 (Fig. 1b), which significantly higher than the other groups. Additionally, PLLA/BTO-GO group also displayed higher expression of Cav1.2, Cav1.3, and Cav1.4 compared to other groups. (Fig.

1c-i). These results implied that the electrical stimulation generated by PLLA/BTO-GO driven by US upregulated the expression of mRNA related to the L-type Ca^{2+} signaling pathway, thus promoting Ca^{2+} influx.

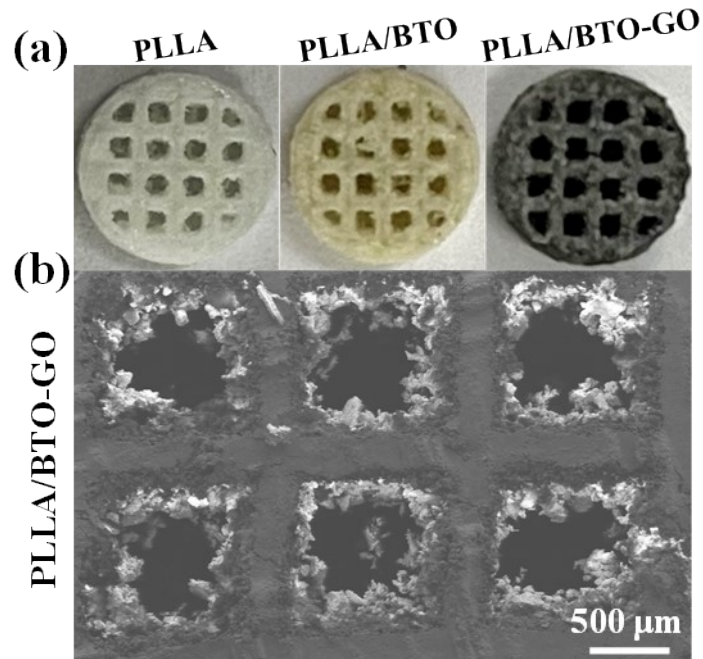


Fig. S1. (a) The macroscopic morphology of PLLA, PLLA/BTO, and PLLA/BTO@GO scaffolds; (b) The microscopic morphology of PLLA/BTO@GO scaffold.

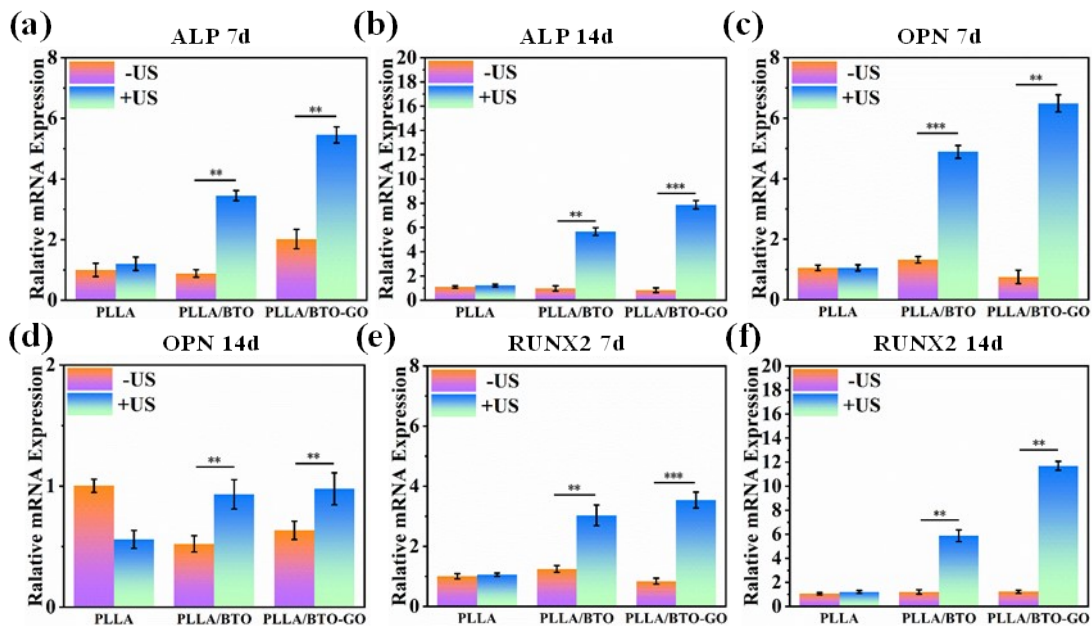


Fig. S2. qPCR analysis of the expression of related genes (a and b) ALP, (c and d) OPN, and (e and f) RUNX2 for mBMSCs cells seeded on different substrates and cultured with or without ultrasound for 7 and 14 days, respectively. * $p = 0.05$, ** $p = 0.01$ and *** $p = 0.001$.

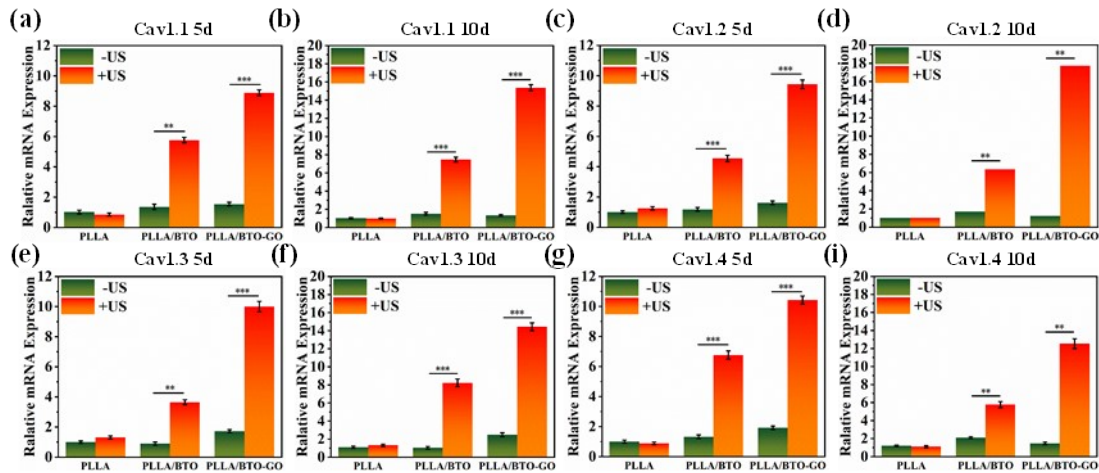


Fig. S3. qPCR analysis of the expression of related genes (a and b) Cav1.1, (c and d) Cav1.2 and (e and f) Cav1.3, Cav1.4 for mBMSCs cells seeded on different substrates and cultured without or with an ultrasound for 5 and 10 days, respectively. * $p = 0.05$, ** $p = 0.01$ and *** $p = 0.001$.