Supporting information

for "Copper-incorporated bioactive glass-ceramics inducing anti-inflammatory phenotype and regeneration of cartilage/bone interface"

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Table S1. The primer sequences used for RT-qPCR analysis

Gene	Forward primer	Reverse primer
GAPDH (rabbit)	5-TCACCATCTTCCAGGAGCGA	5-CACAATGCCGAAGTGGTCGT
COL II	5-AACACTGCCAACGTCCAGAT	5-CTGCAGCACGGTATAGGTGA
ACAN	5-AGGTCGTGGTGAAAGGTGTTG	5-GTAGGTTCTCACGCCAGGGA
SOX-9	5-GGTGCTCAAGGGCTACGACT	5-GGGTGGTCTTTCTTGTGCTG
HIF-1α	5-GCCACCACTGACGATTAAAACC	5-GGTGATGTTGTGGCACTAGC
GAPDH (mouse)	5-TGACCACAGTCCATGCCATC	5-GACGGACACATTGGGGGTAG
TNF-α	5-CTGAACTTCGGGGTGATCGG	5-GGCTTGTCACTCGAATTTTGAGA
IL-18	5-TGGCCGACTTCACTGTACAAC	5-TGGGGTTCACTGGCACTTTG
IL-10	5-GAGAAGCATGGCCCAGAAATC	5-GAGAAATCGATGACAGCGCC
iNOS	5-CAGAAGTGCAAAGTCTCAGACAT	5-GTCATCTTGTATTGTTGGGCT
CD206	5-AGACGAAATCCCTGCTACTG	5-CACCCATTCGAAGGCATTC

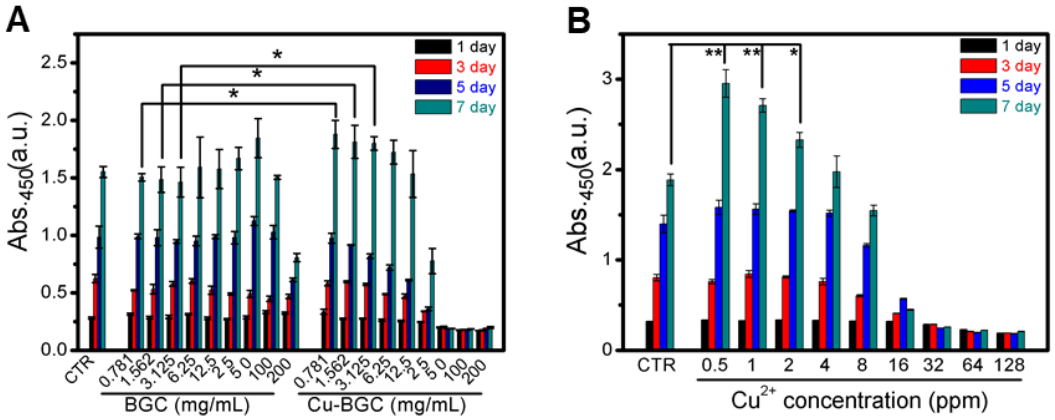

Figure S1. The proliferation of chondrocytes treated with BGC, Cu-BGC ionic extracts and different concentrations of Cu^{2+} ions. As compared with CTR group, the proliferation of chondrocytes treated with different concentrations of Cu-BGC extracts (A) and Cu^{2+} ions (B) was improved within a certain concentration range, respectively. (n=6, *p<0.05, **p<0.01, ***p<0.001)

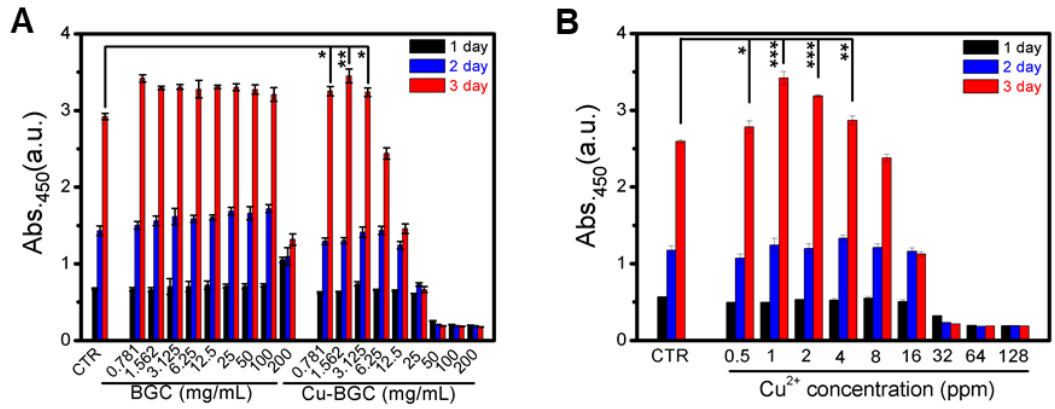

Figure S2. The proliferation of macrophages. The proliferation of macrophages cultured with the ionic products of Cu-BGC (A) and different concentrations of Cu^{2+} ions (B) was enhanced within certain concentrations range, respectively. (n=6, *p<0.05, **p<0.01, ***p<0.001)

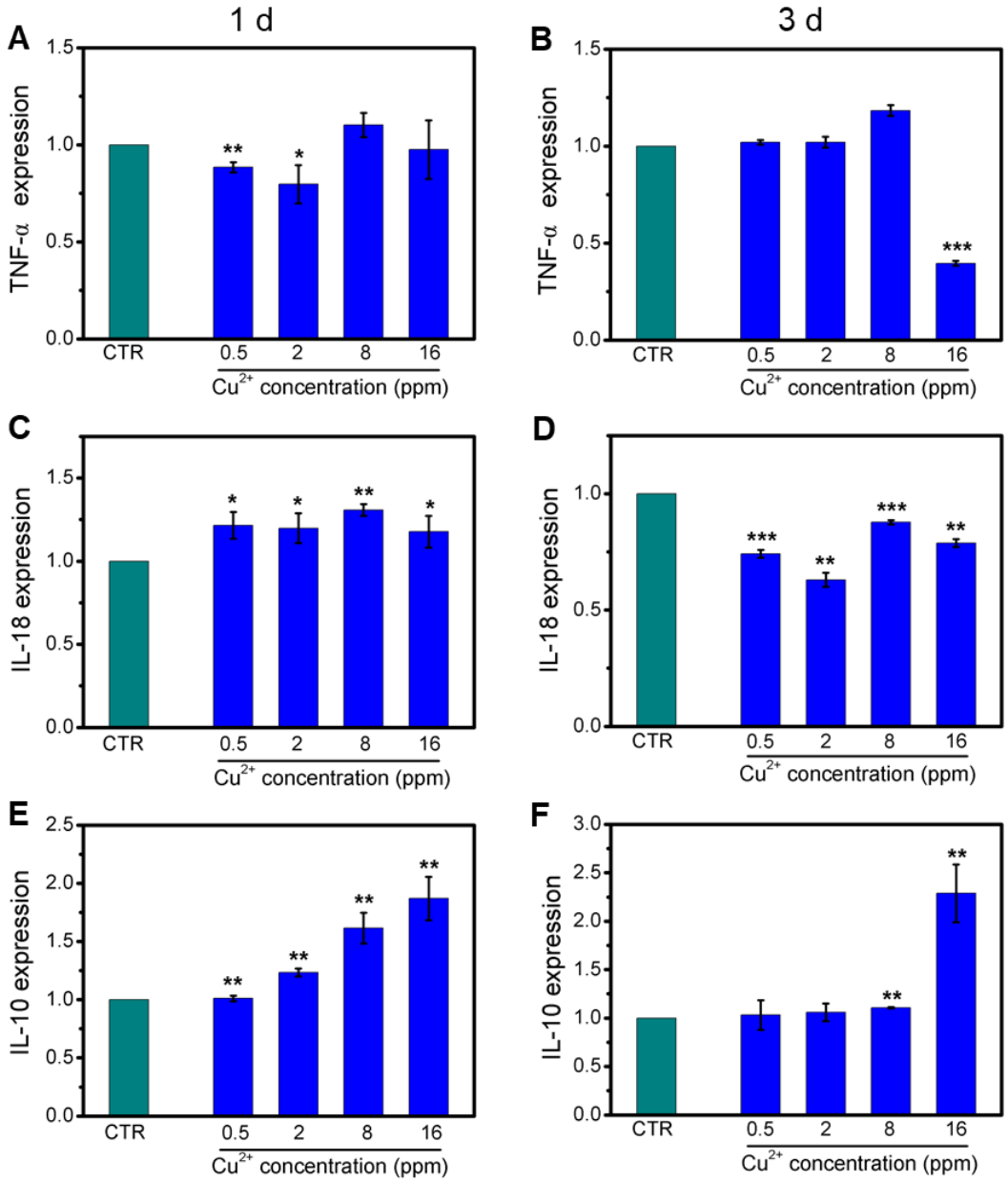

Figure S3. The inflammatory cytokine expression in macrophages stimulated by different concentrations of Cu^{2+} ions. The expression of pro-inflammatory cytokine (TNF-α: A, B; IL-18: C, D) was inhibited, and the anti-inflammatory cytokine (IL-10: E, F) was enhanced after treating with different concentrations of Cu^{2+} ions for 3 days. The relative gene amount of CTR group was set as 1. (n=3, *p<0.05, **p<0.01, ***p<0.001)

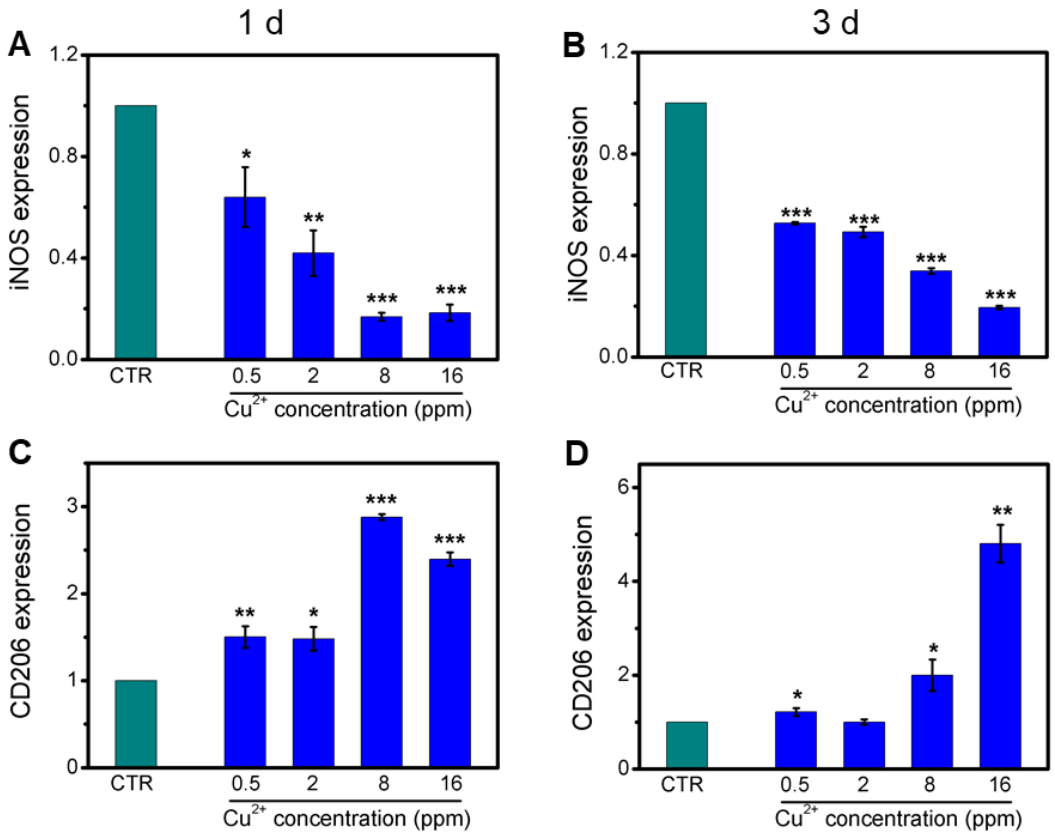

Figure S4. The expression of macrophage surface markers in macrophage after treating with different concentrations of Cu^{2+} ions at day 1 and 3. (A, B) M1 marker: iNOS; (C, D) M2 marker: CD206. The macrophage phenotype was changed to an anti-inflammatory M2 phenotype after treating with different concentrations of Cu^{2+} ions. The relative gene amount of CTR group was set as 1. (n=3, *p<0.05, **p<0.01, ***p<0.001)

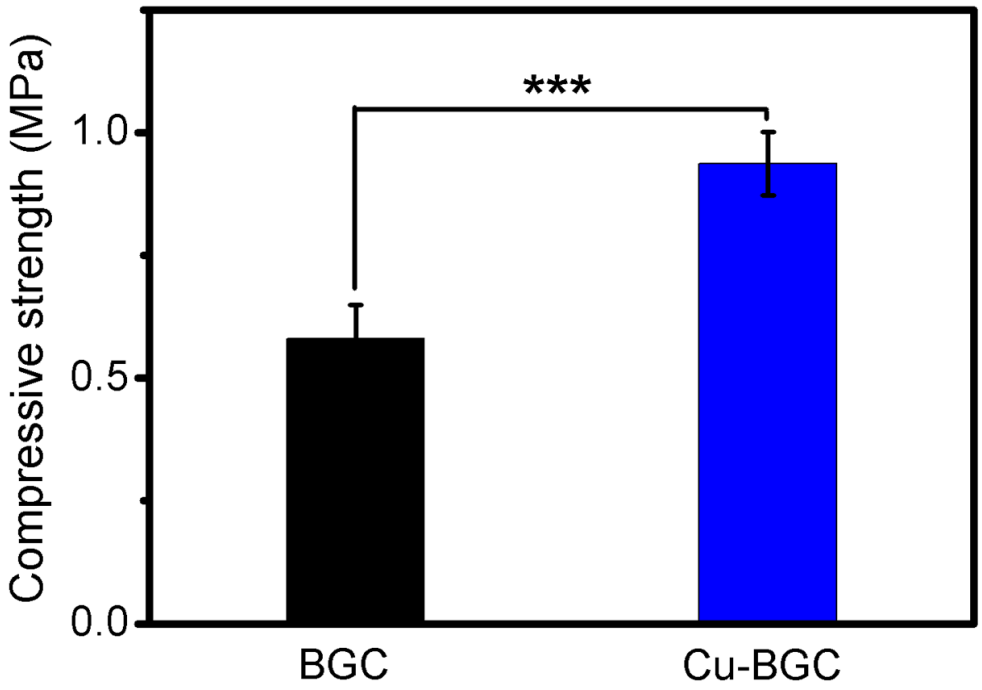

Figure S5. The compressive strength of BGC and Cu-BGC scaffolds. (A) BGC scaffold, (B) Cu-BGC scaffold. The results indicated that Cu-BGC scaffolds possess a higher compressive strength as compared with BGC scaffolds. (n=6, *p<0.05, **p<0.01, ***p<0.001)

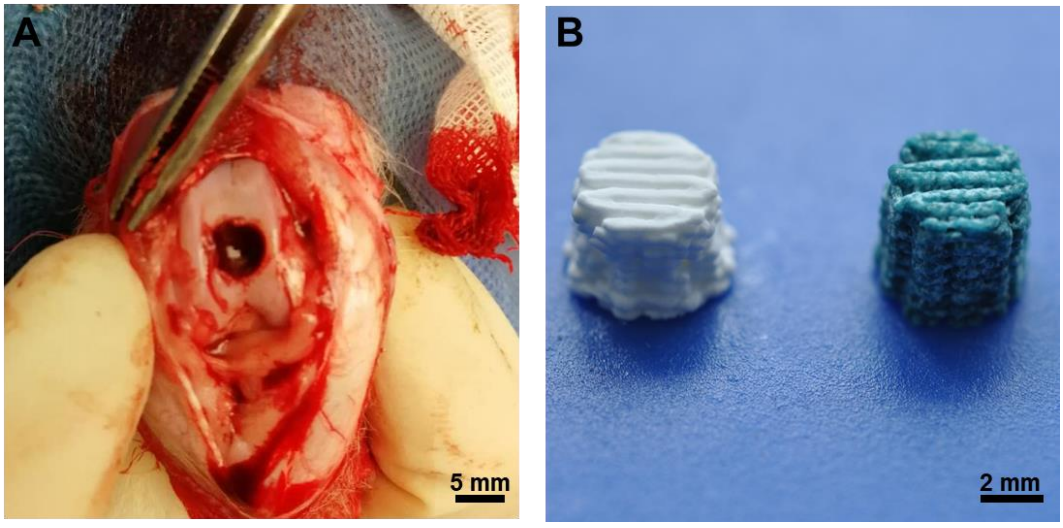

Figure S6. The digital photographs of the defect and the scaffolds during the surgery. (A) The osteochondral defect was 5 mm high and 5 mm in diameter. (B) The size of scaffolds which were implanted into the defects was 5 mm high and 5 mm in diameter.

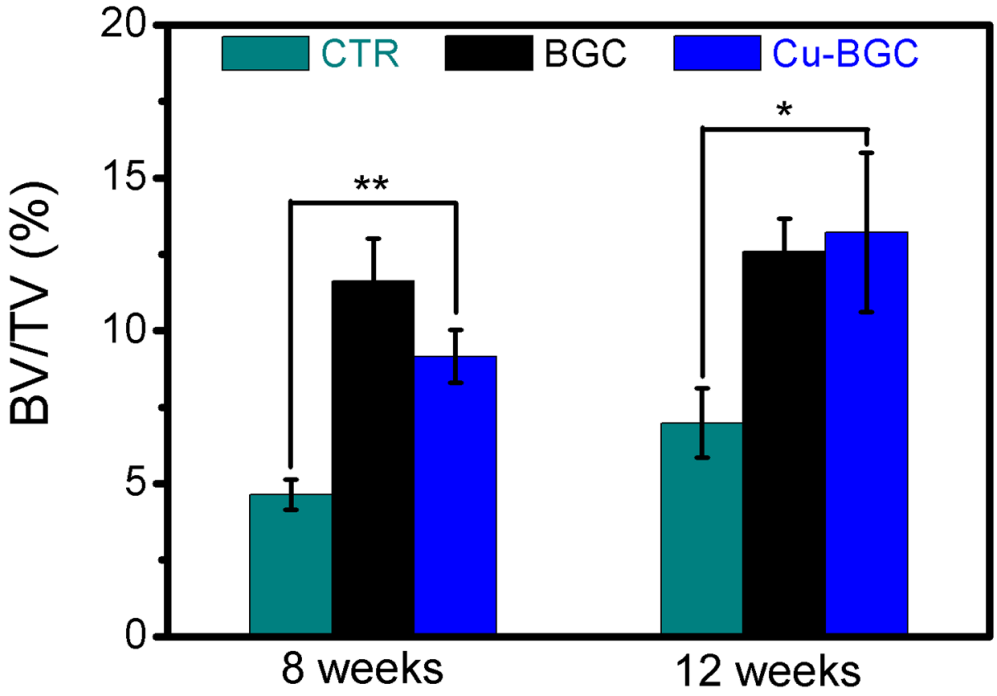

Figure S7. Micro-CT imaging analysis (BV/TV) of the defects at 8 and 12 weeks. Micro-CT analysis of defect space showed that it was greater level of bone formation in Cu-BGC group as compared to CTR group. (n=6, *p<0.05, **p<0.01, ***p<0.001)

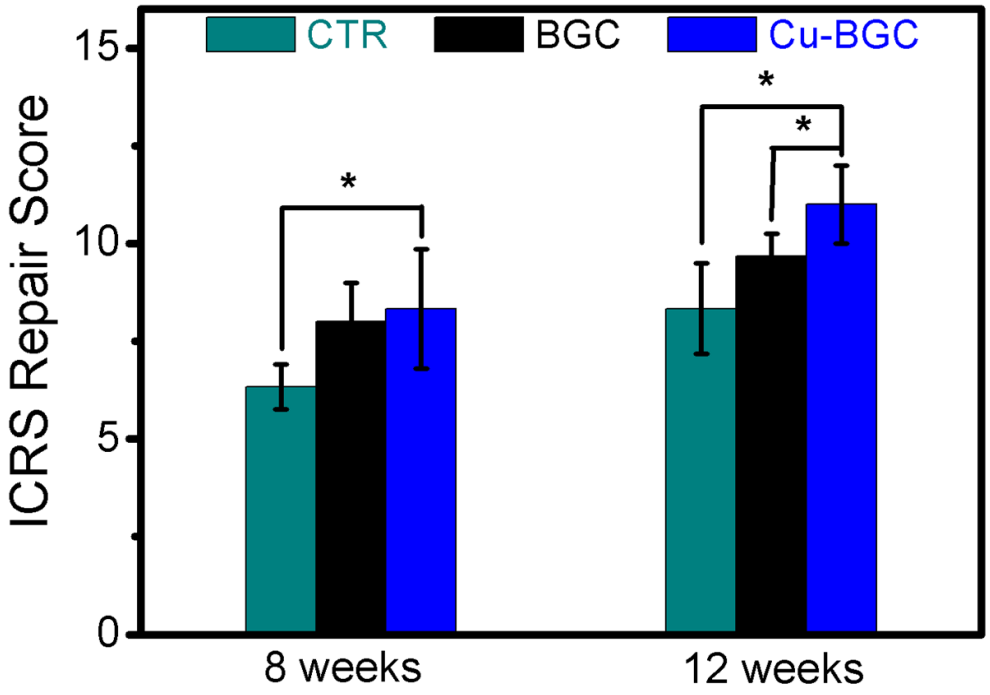

Figure S8. The ICRS scores of knee samples at 8 and 12 weeks of postsurgery. Blinded quantity of ICRS scores for CTR, BGC and Cu-BGC groups by three investigators, the results showed that the ICRS score of Cu-BGC group was enhanced as compared to CTR and BGC groups at 12 weeks. (n=6, *p<0.05, **p<0.01, ***p<0.001)