Supporting Information

Reduced Graphene Oxide-Reinforced Natural Calcium Phosphate Cements for Bone Tissue Engineering

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Experimental Section

Energy-Dispersive X-Ray Spectroscopy (EDS)

The points of study were determined and analyzed with EDS mounted in FESEM (SUPRA 55VP, Carl Zeiss, Germany). This resulted in a histogram, where the horizontal axis displays units of energy [kilo electron volts (keV)] and the vertical axis represent the intensity.

Soaking Test

To perform the bioactivity test, simulated body fluid (SBF) was prepared by dissolving NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H2O$, $MgCl_2 \cdot 6H2O$, and Na_2SO_4 in distilled water and HCl to reach a pH value of 7.4, following the protocol of Kokubo et al[45]. Each bone cement specimen was immersed in 500 µl of SBF for 7, 14, or 21 days at 37℃ and 100% humidity. The SBF was replaced with fresh SBF every week. After the due date, each specimen was collected and dried at 37℃ and 100% humidity. The dried samples were weighed, and their mechanical properties were analyzed by texture analyzer.

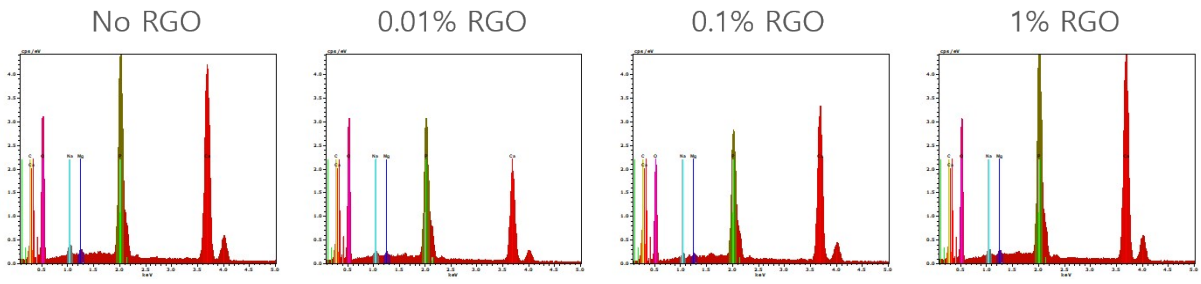

Figure S1. EDS analysis results. An existence of calcium and phosphate elements was confirmed. EDS: energy-dispersive X-ray spectroscopy

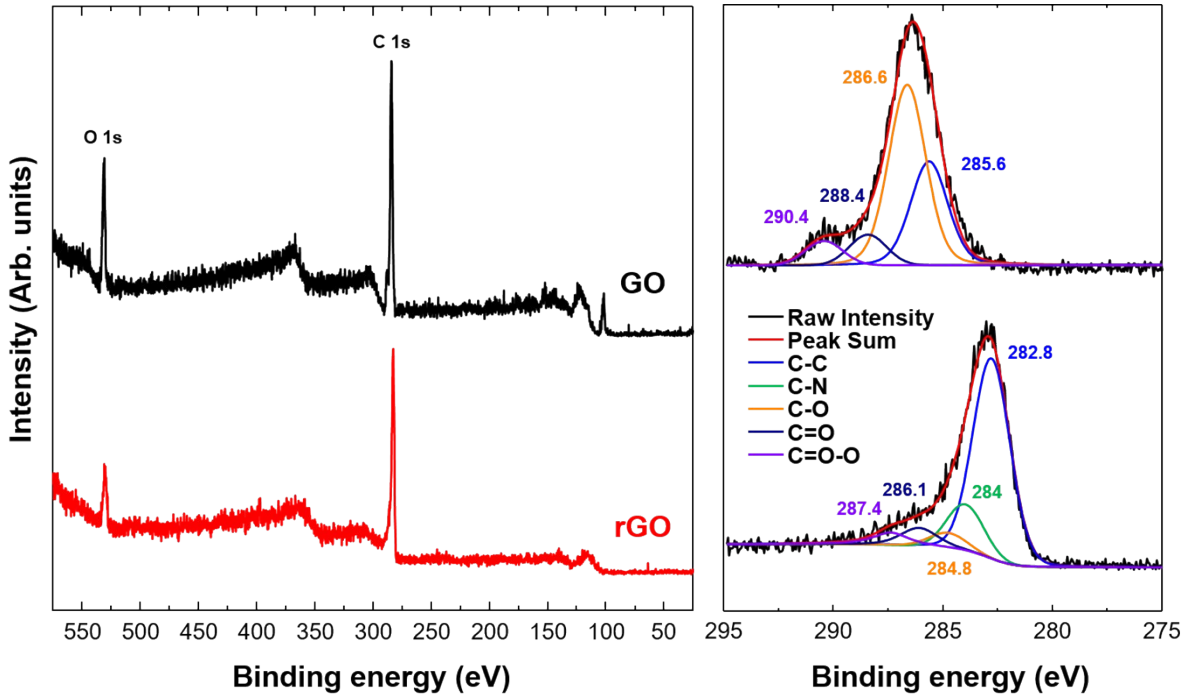

Figure S2. XPS results of GO and RGO. O1s peak of GO decreased compared to RGO. Whereas, C-C bonding in C1s peak increased. XPS: X-ray photoelectron spectroscopy, GO: graphene oxide, RGO: reduced graphene oxide.

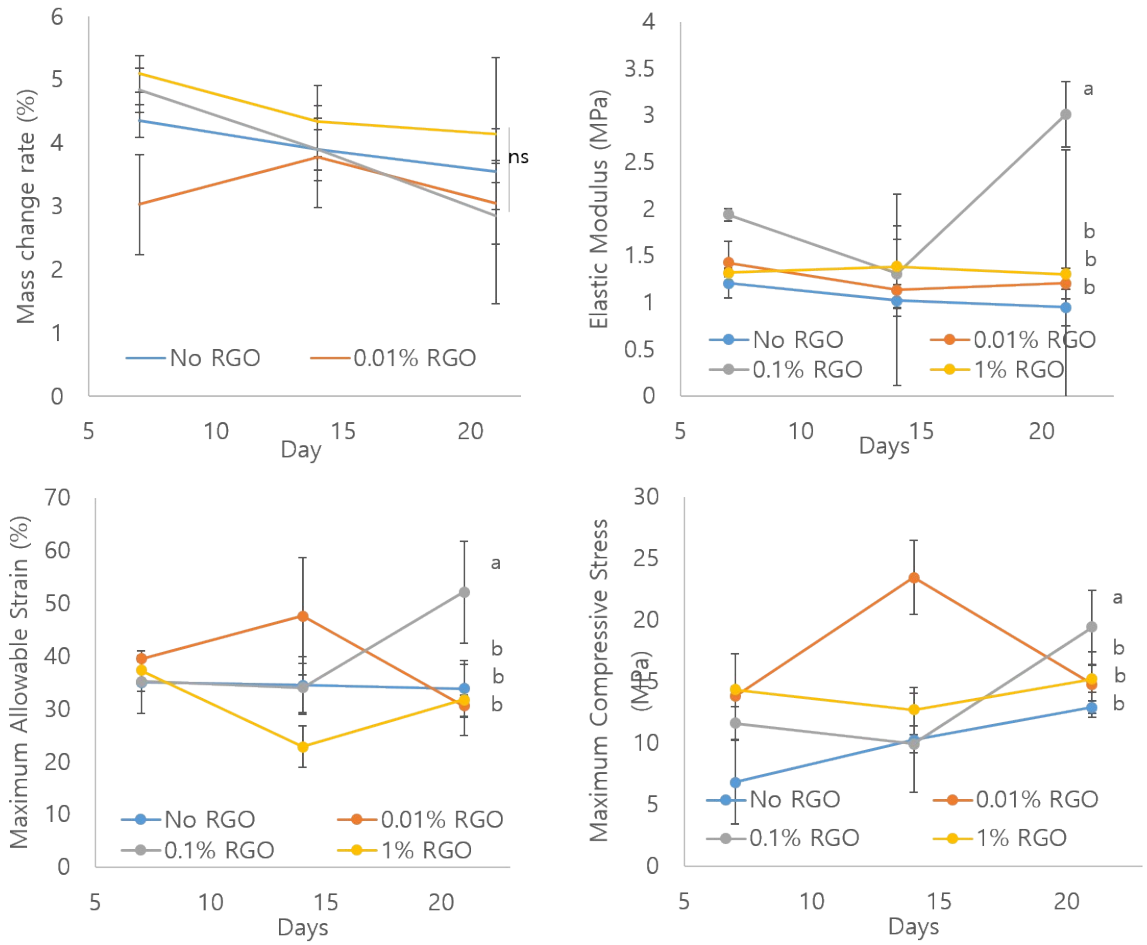

Figure S3. Soaking study of RGO-CPCs. The mass change rate, elastic modulus, maximum allowable strain, and maximum compressive stress were investigated for 3 weeks. The elastic modulus, maximum allowable strain, and maximum compressive stress of 0.1% RGO were significantly improved in comparison with the other samples. (n = 5, LSD)

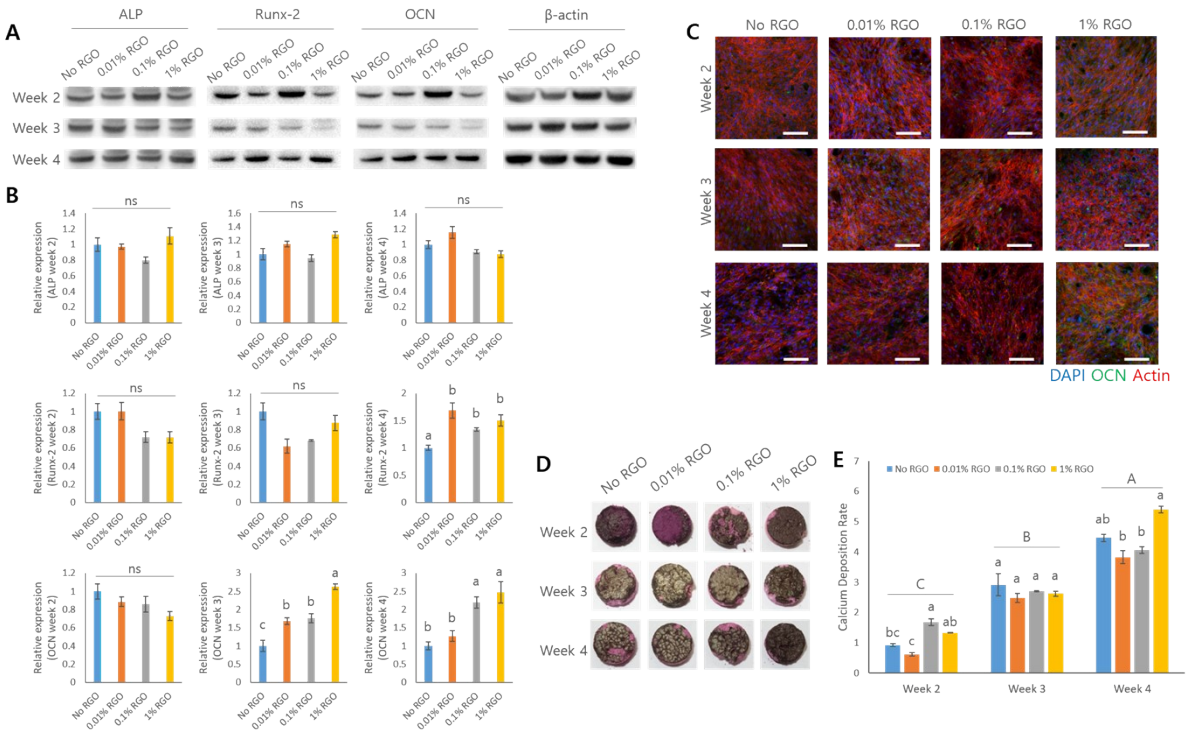

Figure S4. Osteogenic differentiation study of MC3T3-E1 (A) Representative western blot result. ALP, Runx-2, OCN, and β-actin expressions were evaluated during 4 weeks. (B) The relative expressions of osteogenic marker protein were calculated. The expression of ALP was not significantly different during 4 weeks. The expression of Runx-2 and OCN showed discrete differences in week 4. (C) ICC results. OCN expression for 4 weeks is depicted. At 4 weeks, 1% RGO had the highest OCN expression. Scale bars = 100 μm. (D) Representative images of ARS. (E) Quantitative analysis of ARS. The calcium deposition rate of 1% RGO in week 4 showed the highest value with significant difference. (n = 5, LSD) ALP: alkaline phosphatase, Runx-2: runt-related transcription factor-2, OCN: osteocalcin, ICC: immunocytochemistry, ARS: alizarin red staining

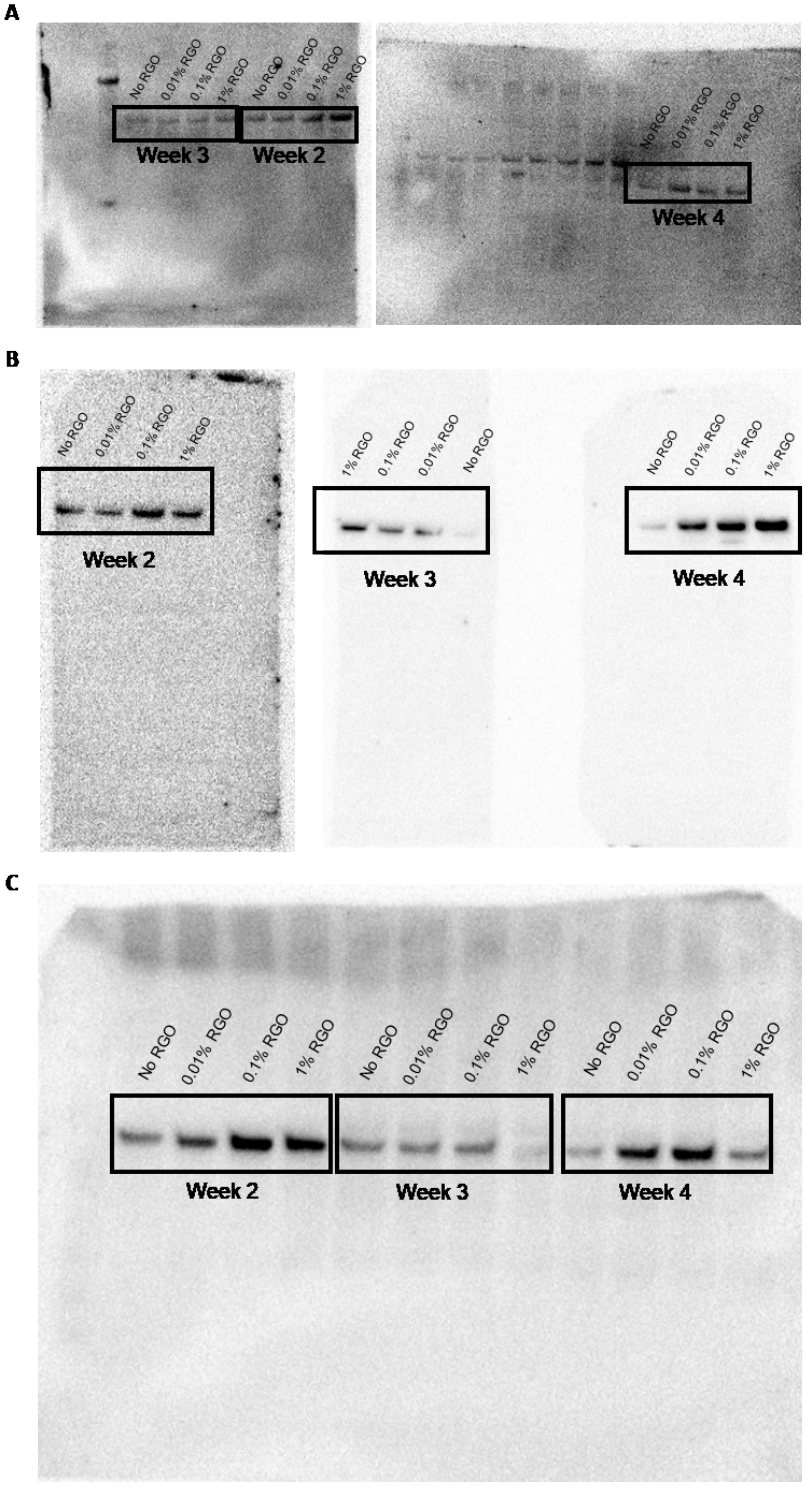

Figure S5. Osteogenic differentiation study of cells used in this study. Full gel images of the representative western blot results. (A)ALP, (B)OCN and (C)β-actin expression of rASC were evaluated for 4 weeks.

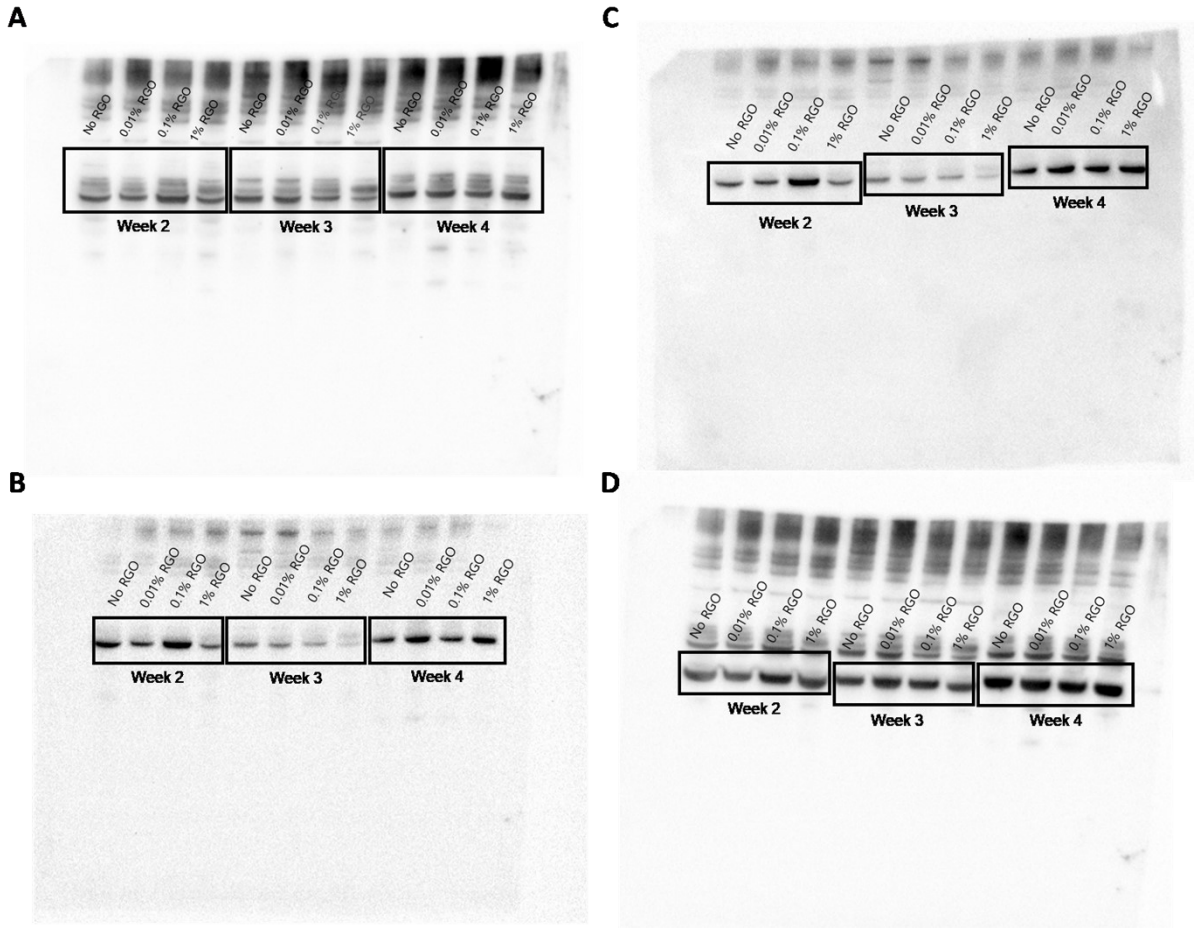

Figure S6. Osteogenic differentiation study of cells used in this study. Full gel images of the representative western blot results. (A)ALP, (B)RUNX-2, (C)OCN and (D)β-actin expression of MC3T3-E1 was evaluated for 4 weeks.