

Supplementary material

The Effect of Low-Temperature Thermal Processing on Bovine Hydroxyapatite Bone Substitutes, toward Bone Cell Interaction and Differentiation

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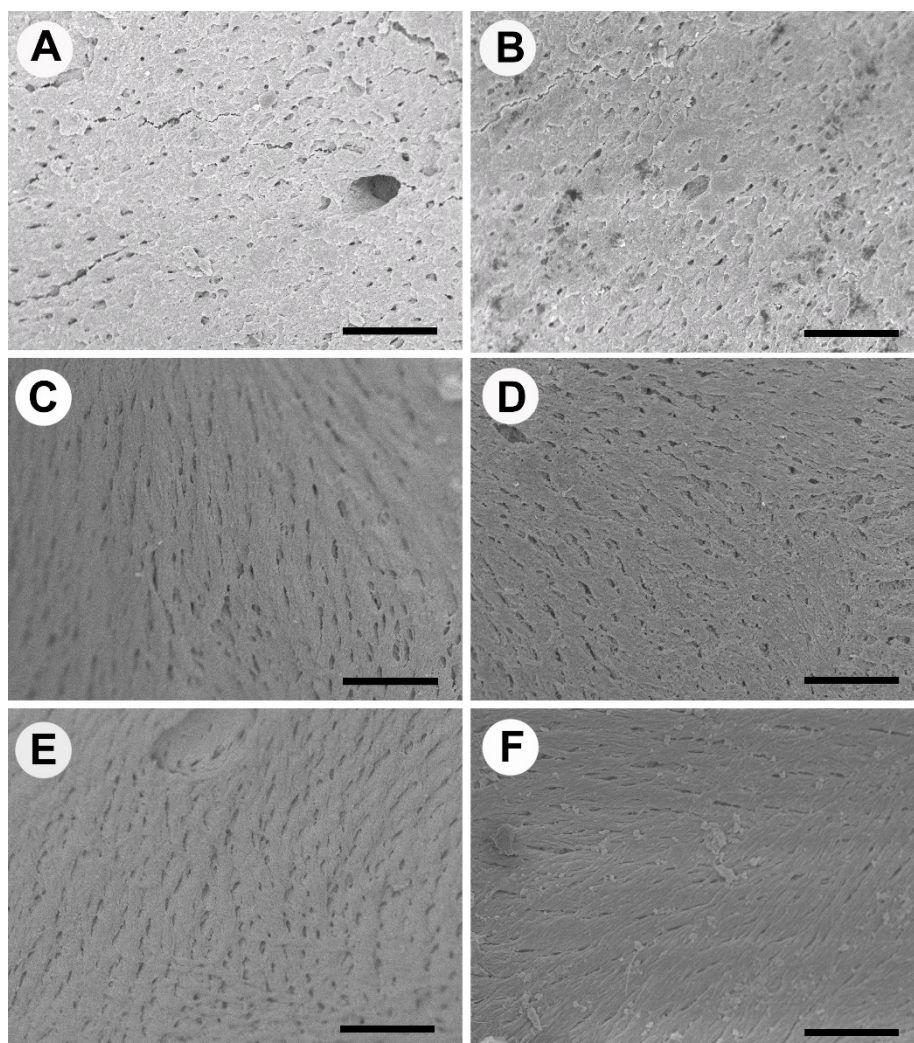


Figure S1. Scanning electron microscopy images of the surfaces of bone scaffolds heat-treated at (A) 100 °C, (B) 130 °C, (C) 160 °C, (D) 190 °C, (E) 220 °C, (F) Bio-Oss®. Scale bar = 10 µm. Representative images of N=4.

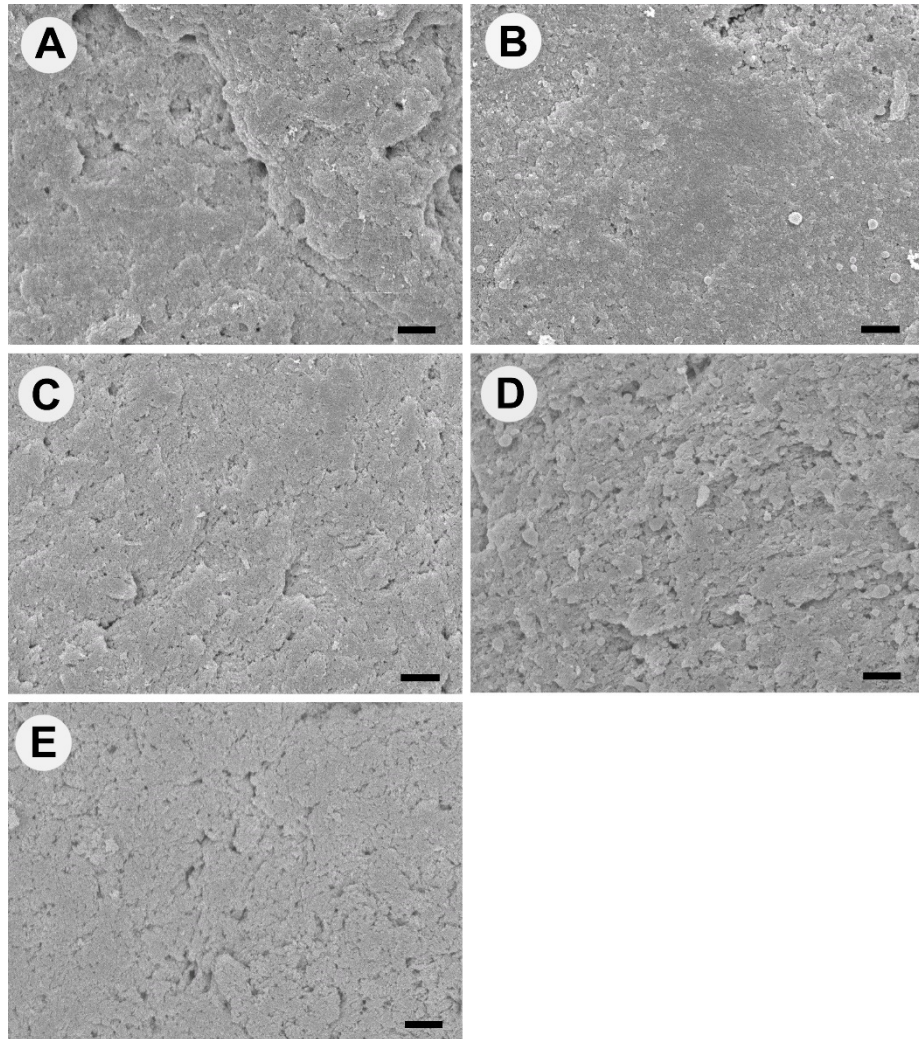


Figure S2. Scanning electron microscopy images of the cut surfaces of bone scaffolds heat-treated at (A) 100 °C, (B) 130 °C, (C) 160 °C, (D) 190 °C, (E) 220 °C. Scale bar = 1 μ m. Representative images of N = 4.

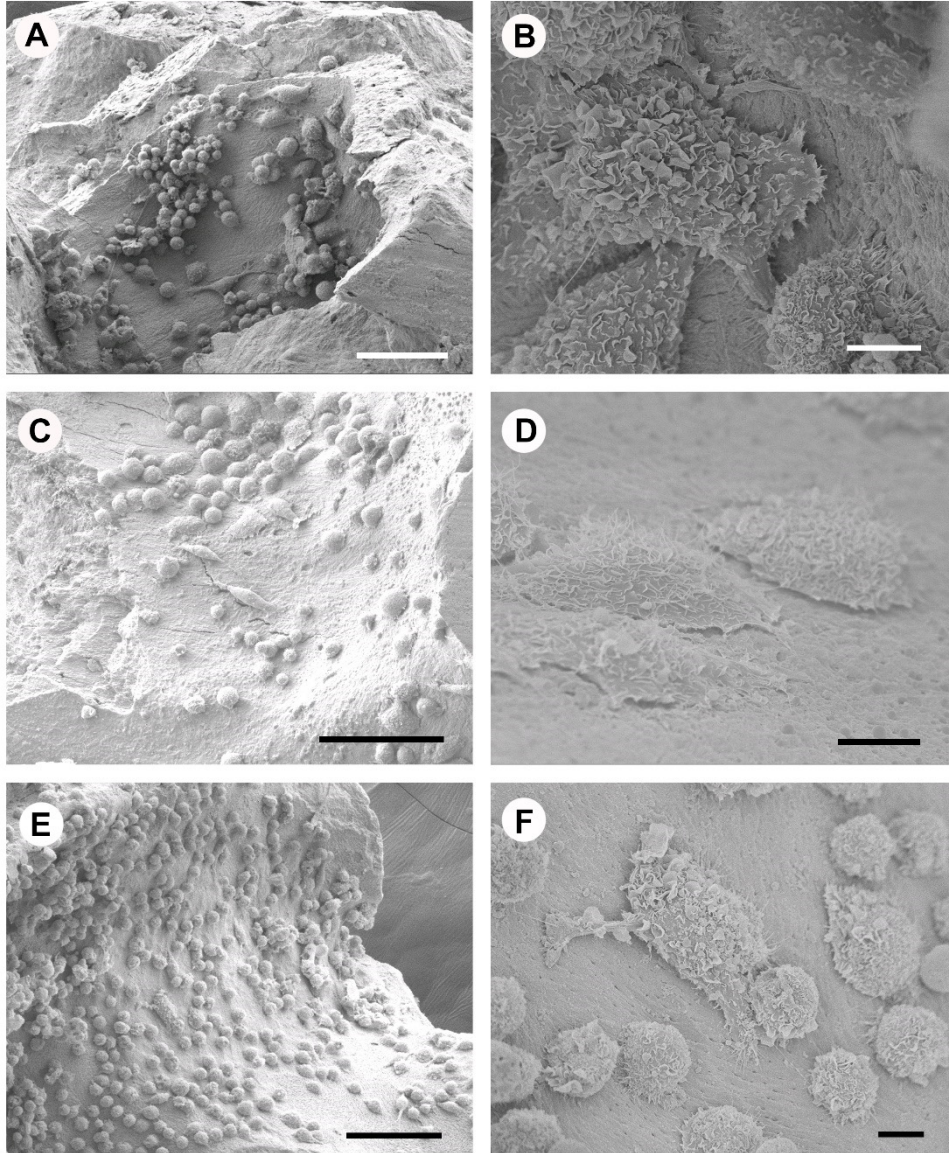


Figure S3. High magnification scanning electron microscopy images of osteoclasts cultured on bone scaffold specimens heat-treated at 100 °C (**A,B**) 130 °C (**C,D**) and Bio-Oss® (**E,F**). Scale bar =100 μ m (left) and 10 μ m (right). N = 4.

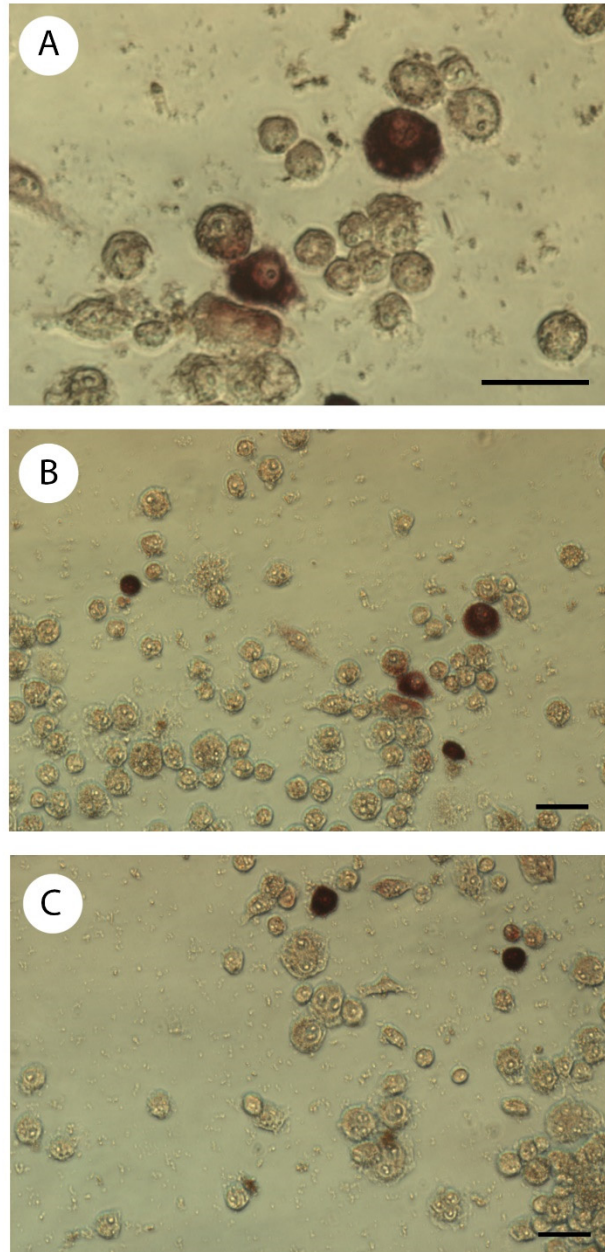


Figure S4. Light microscopy images showing tartrate-resistant acid phosphatase positive and multinucleated osteoclasts (pink) on plastic wells at **(A)** high magnification and **(B,C)** duplicate wells. Scale bar = 50 μm .

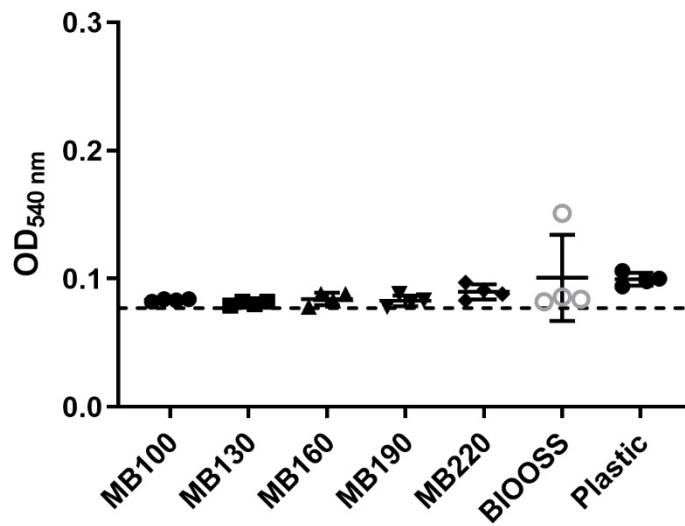


Figure S5. Tartrate-resistant acid phosphatase activity determined from the supernatant of osteoclasts cultured on bone scaffold specimens at 7 days. N = 4. Results expressed as mean $\pm$ SD. Dotted line represents the negative control value.

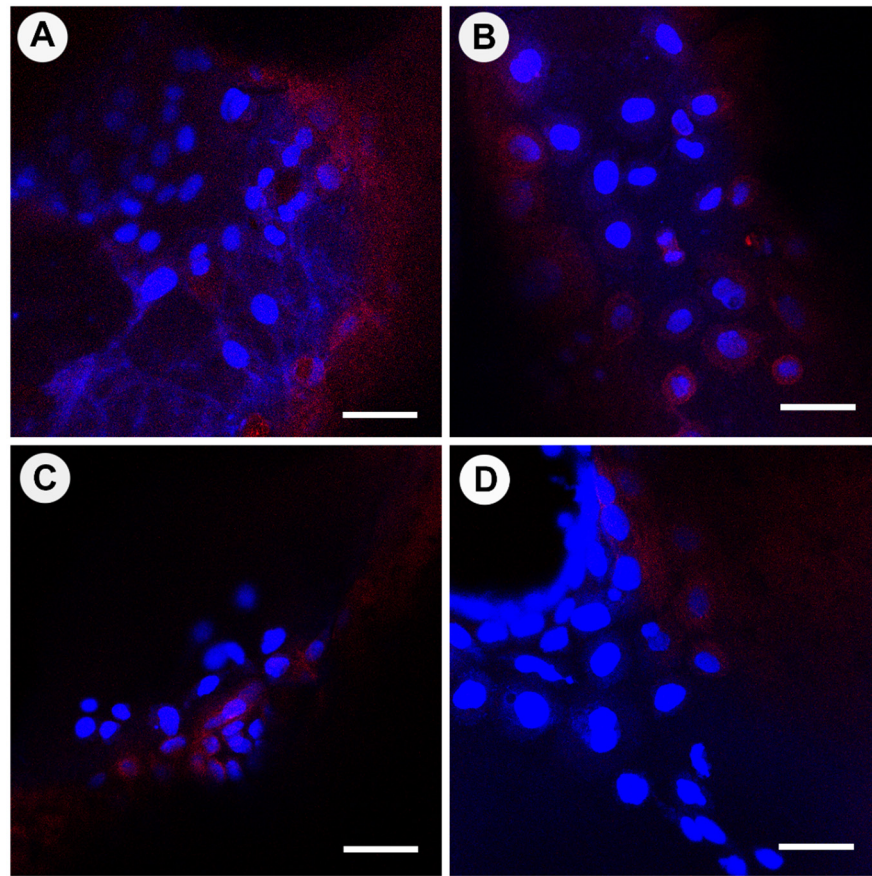


Figure S6. Confocal laser scanning microscopy (CLSM) of osteoblast actin filaments (red) and osteoblast nuclei (blue) cultured in non-osteogenic media for 21 days on bone scaffolds heat-treated at (A) 100 °C, (B) 130 °C, (C) 160 °C, and (D) BioOss. Scale bar = 50 μm.

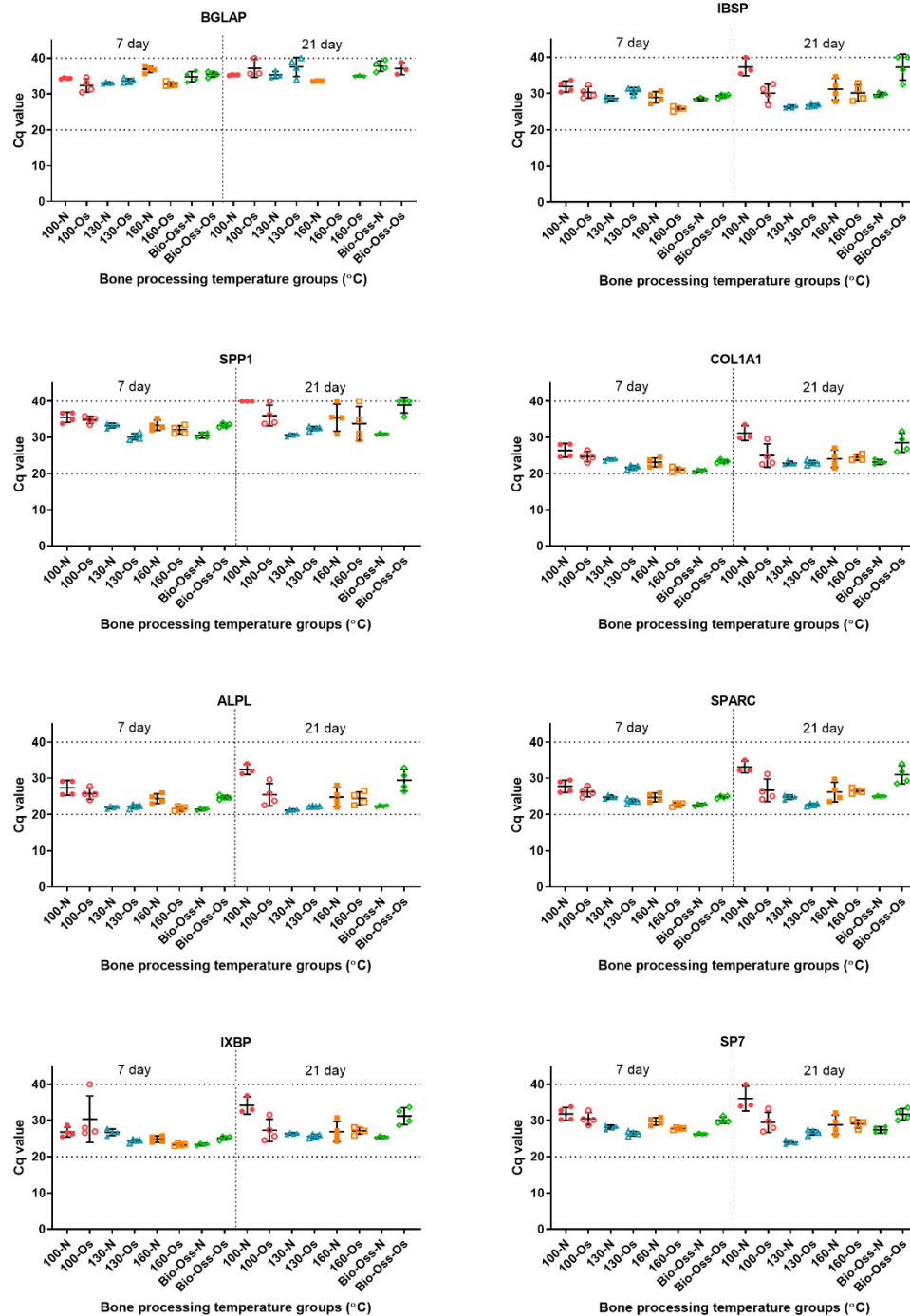


Figure S7. Cq values of genes of interest expressed in osteoblasts (Saos-2 cells) cultured on bone processed at different temperatures at 7 and 21 days, in non-osteogenic (-N) and osteogenic media (Os). N= 4. Results expressed as mean $\pm$ SD. Dotted line at Cq 40 = not detected, dotted line at Cq 20 = highly expressed.