

Supplemental Information

Bioactive Nanoengineered Hydrogels for Bone Tissue Engineering: A Growth-Factor-Free Approach

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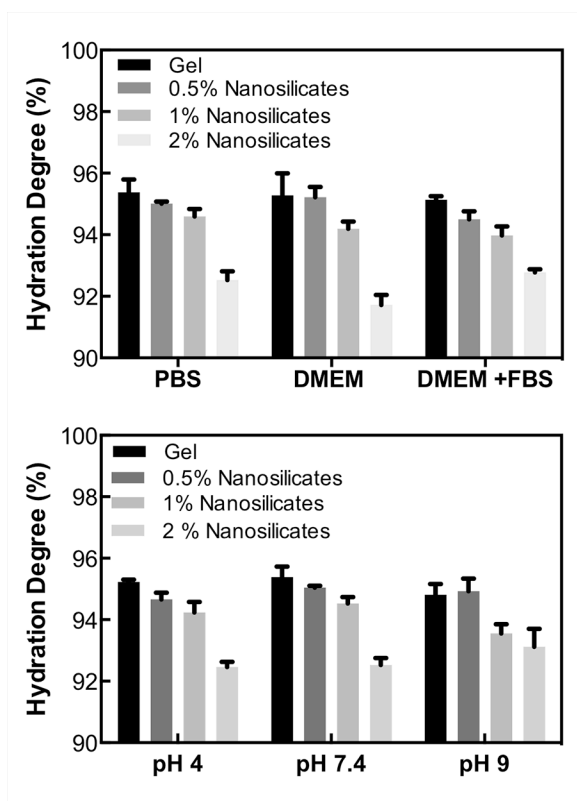


Figure S1: Effect of nanosilicates on *in vitro* stability of nanocomposite. The hydration degree of Gel and nanocomposites was determined at media conditions (PBS, DMEM, and DMEM+FBS) and different pH (4, 7.4, and 9).

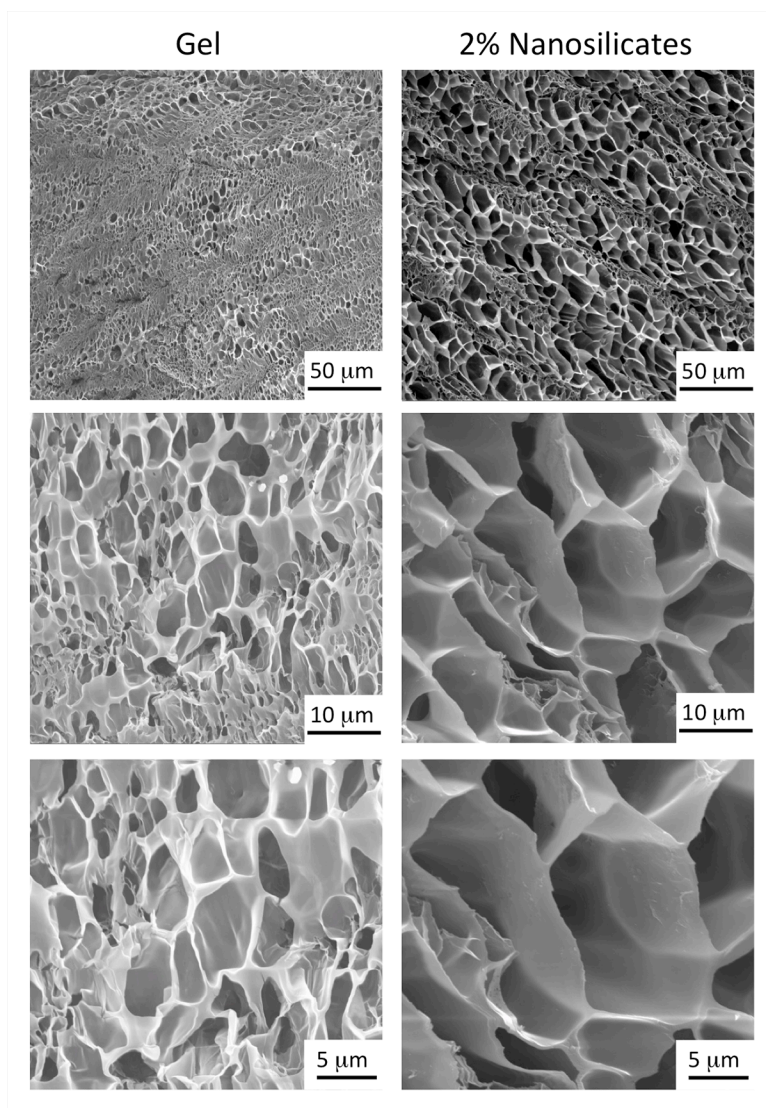


Figure S2: Effect of nanosilicates on surface morphology of nanocomposite hydrogels. The nanocomposite hydrogels exhibited a highly porous and interconnected network. The silicate nanoparticles were uniformly distributed within the polymeric network, as indicated by a lack of nanoparticle aggregation or phase separation.

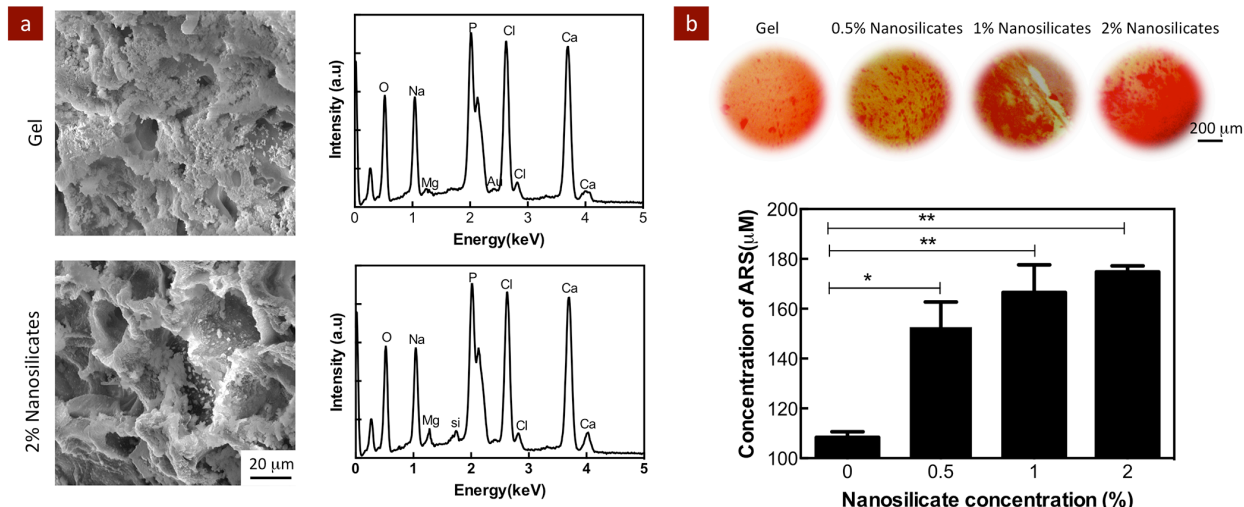


Figure S3: The bioactivity of the nanocomposites was investigated by submerging the hydrogels in stimulated body fluid (SBF). (a) Both Gel and nanocomposites showed bioactivity in the presence of SBF, as determined by the presence of Ca and P peaks in the EDX spectra indicative of mineralized structure. (b) The amount of the mineralized matrix deposited was determined using Alizarin Red S staining and quantification. The addition of nanosilicates significantly promoted deposition of the mineralized matrix (stained red). The quantification of deposited calcium also indicated that addition of nanosilicates resulted in a more than 50% increase in the mineralized matrix deposition (* $p < 0.05$, ** $p < 0.01$).

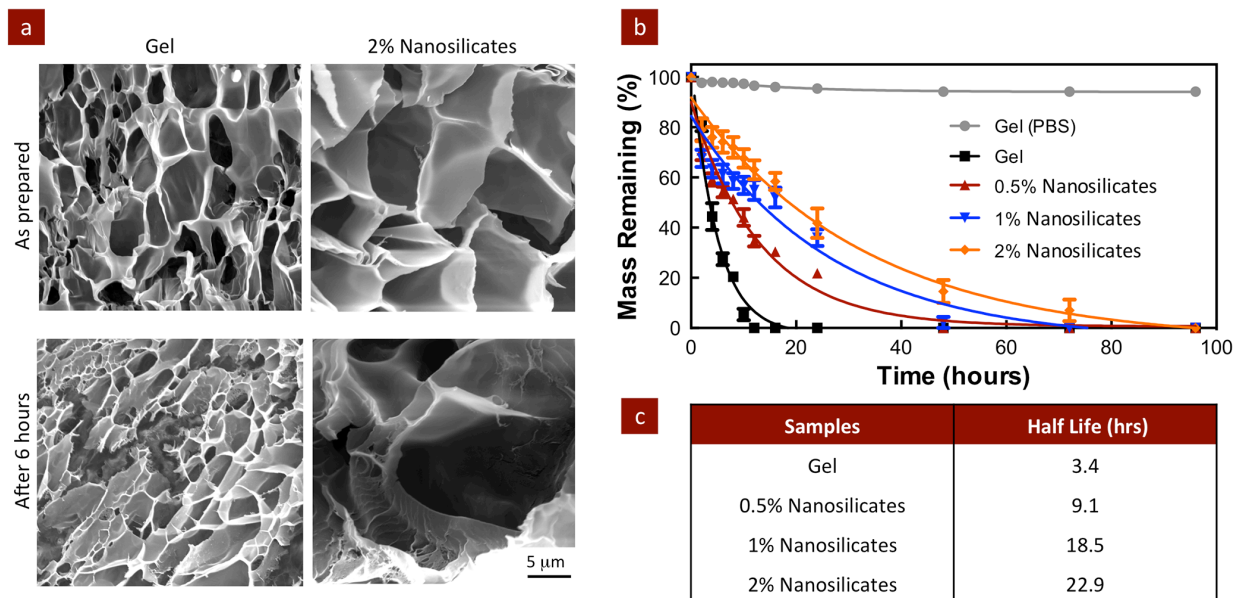


Figure S4: Effect of nanosilicates on *in vitro* stability of nanocomposite. (a) The hydration degree of Gel and Gel-nanosilicate was determined at different pH (4, 7.4, and 9) and media conditions (PBS, DMEM, and DMEM+FBS). (b) The effect of nanosilicates on the enzymatic degradation of the hydrogel network was evaluated in a collagenase solution at 37°C. (c) The data are summarized to extract half-life values by fitting the data to a first-order degradation model.

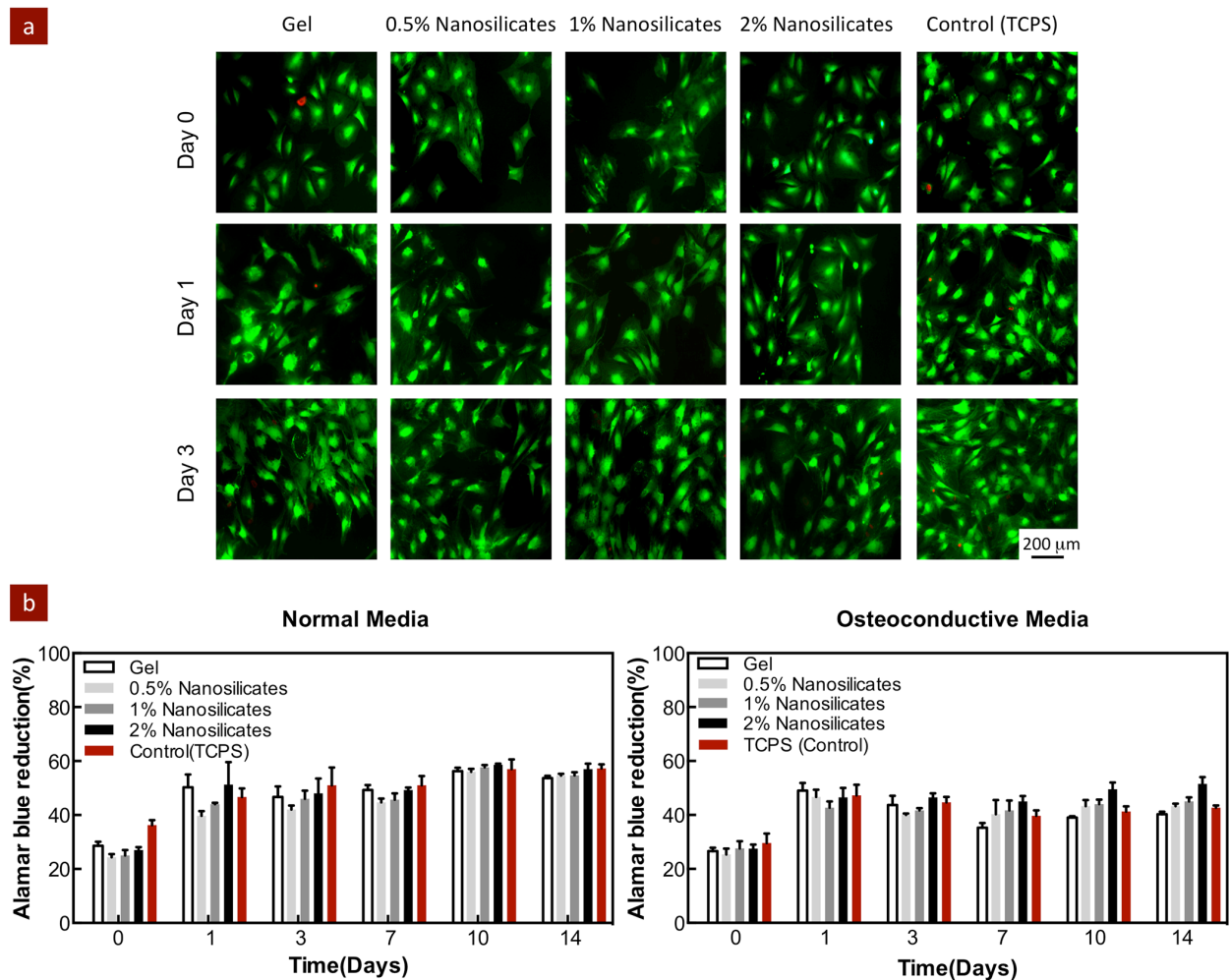


Figure S5: *In vitro* cell adhesion and proliferation of preosteoblasts on Gel and nanocomposite hydrogels. (a) All hydrogels supported initial cell adhesion and spreading, as determined by live/dead imaging of cells seeded on hydrogel surfaces on Day 0 (3 hours), Day 1, and Day 3. TCPS acted as the positive control. (b) The metabolic activity of seeded cells was monitored using an Alamar Blue assay. Cells seeded on all the hydrogel surfaces proliferated over a period of 14 days in normal and osteoconductive media.

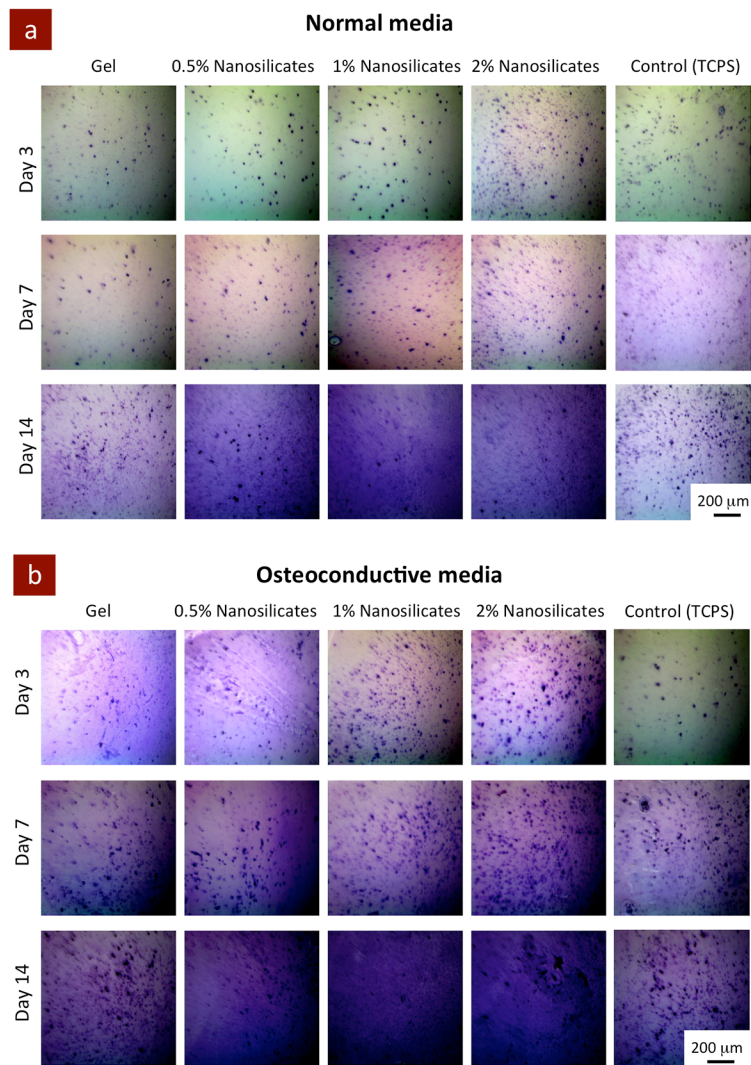


Figure S6: Effect of nanosilicates on ALP activity in preosteoblast cells. Representative images are shown for cultures stained with NBIT/BCIP for intercellular ALP on Day 3, 7, and 14 in (a) normal growth media and (b) osteoconductive media. The number of ALP activity cells (purple stain) increased with an increase in time (from Day 3 to Day 14).

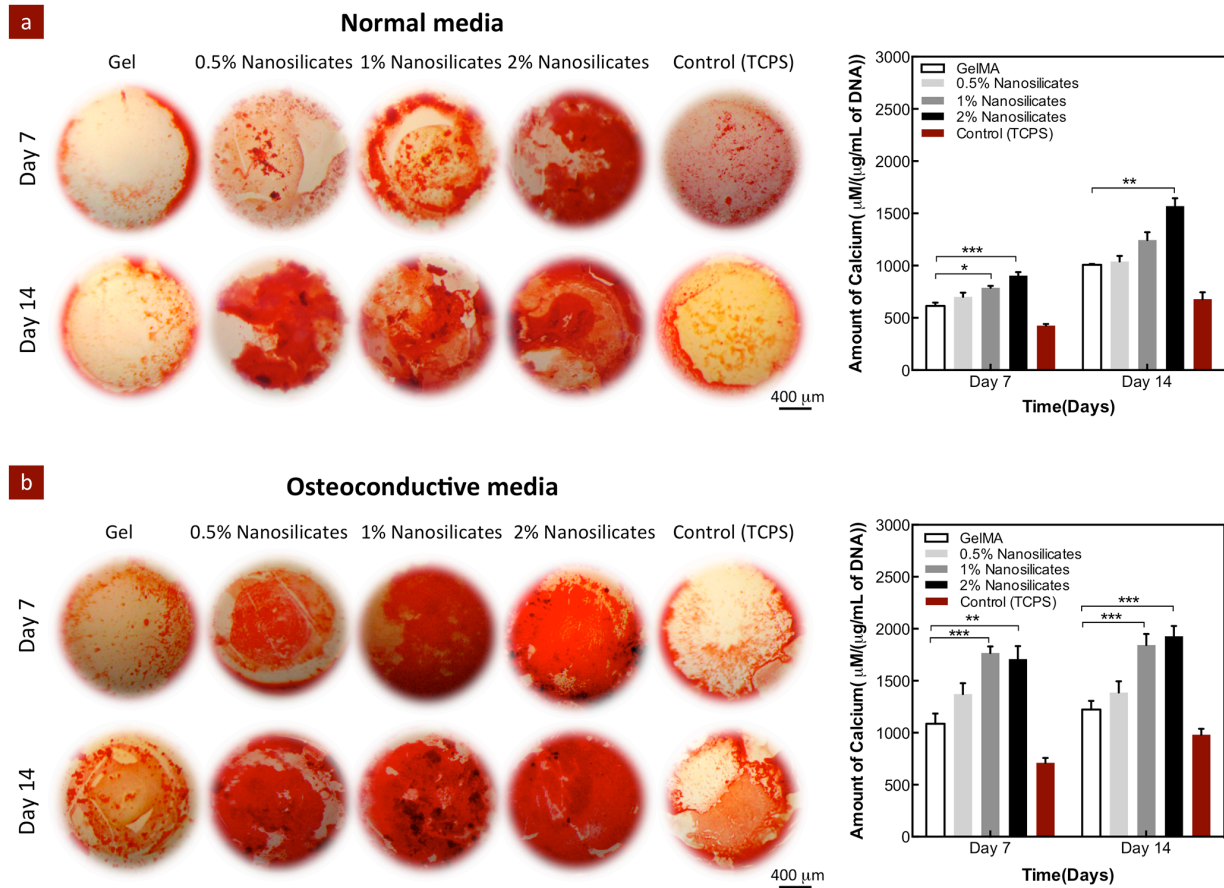


Figure S7: The effect of nanosilicates on the production of the mineralized ECM was evaluated. Inorganic calcium deposited by preosteoblast cells in (a) normal and (b) osteoconductive media was identified using Alizarin Red S staining. The optical images show the deposition of the mineralized matrix (stained red) on Days 7 and 14). The amount of calcium deposited was quantified from ARS stained sample and normalized with DNA content.

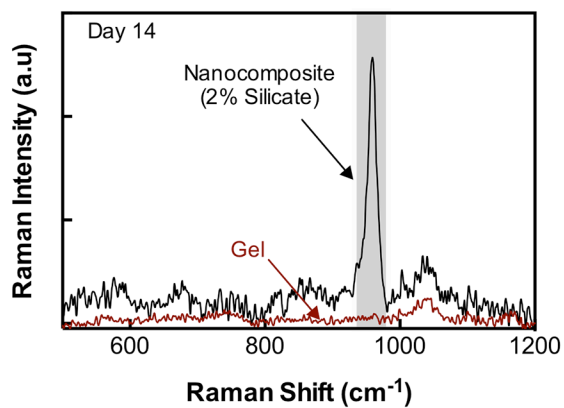


Figure S8: The Raman spectrum of Gel and Gel-2%Silicate hydrogels after Day 14 in osteoconductive media. The presence of a very strong PO_4 peak at 961 cm^{-1} , indicate presence of hydroxyapatite in nanocomposite hydrogels.