Supporting Information

Inspired by nature: facile design of nanoclay-organic hydrogel bone sealant with multifunctional properties for robust bone regeneration

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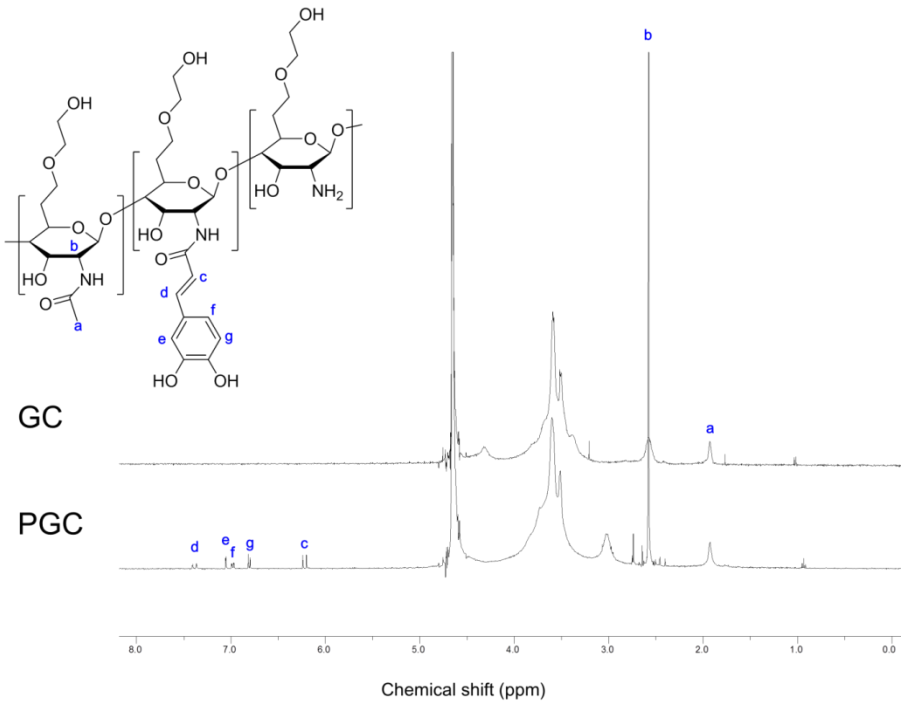


Figure S1. ^{1}H NMR spectra of glycol chitosan (GC) and phytochemical conjugated GC (PGC) in D_2O.

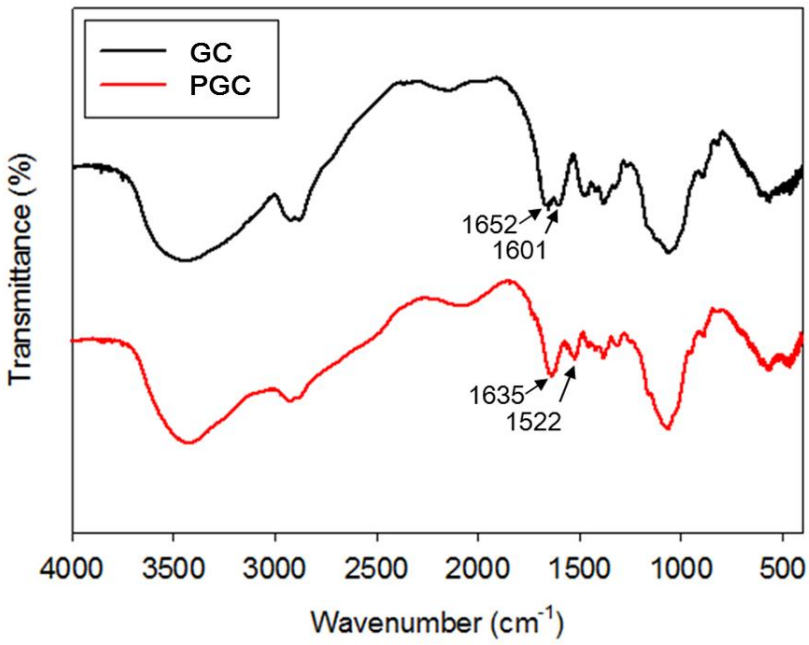


Figure S2. FT-IR spectra of GC and PGC.

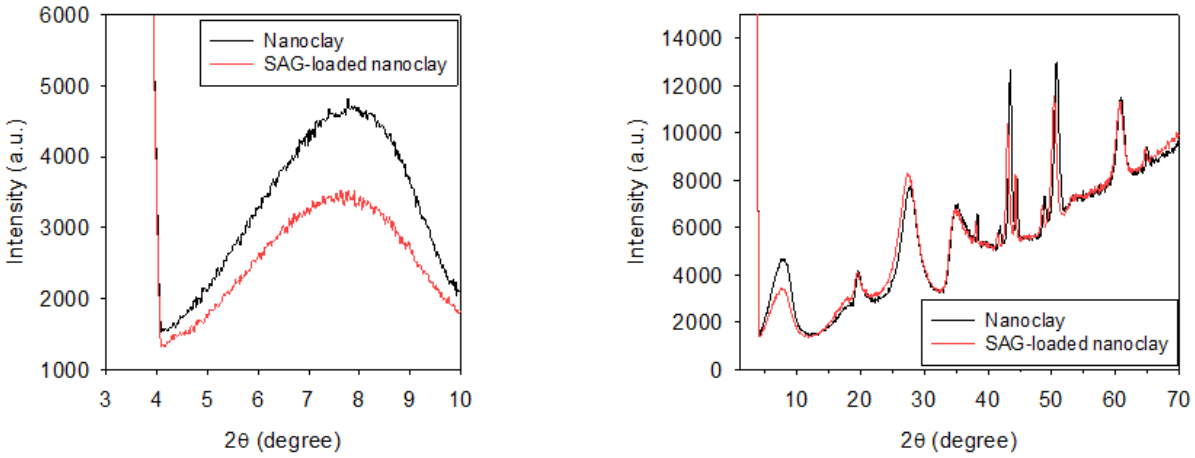


Figure S3. XRD patterns of nanoclay and SAG-loaded nanoclay.

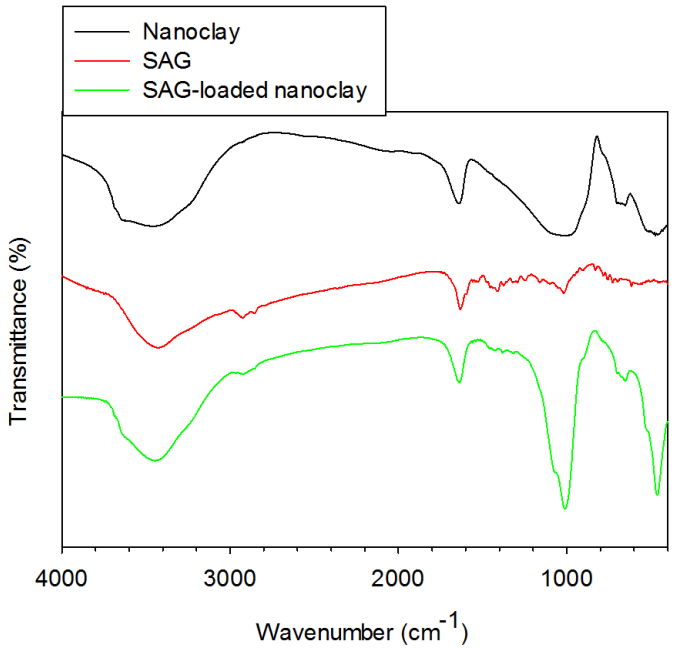
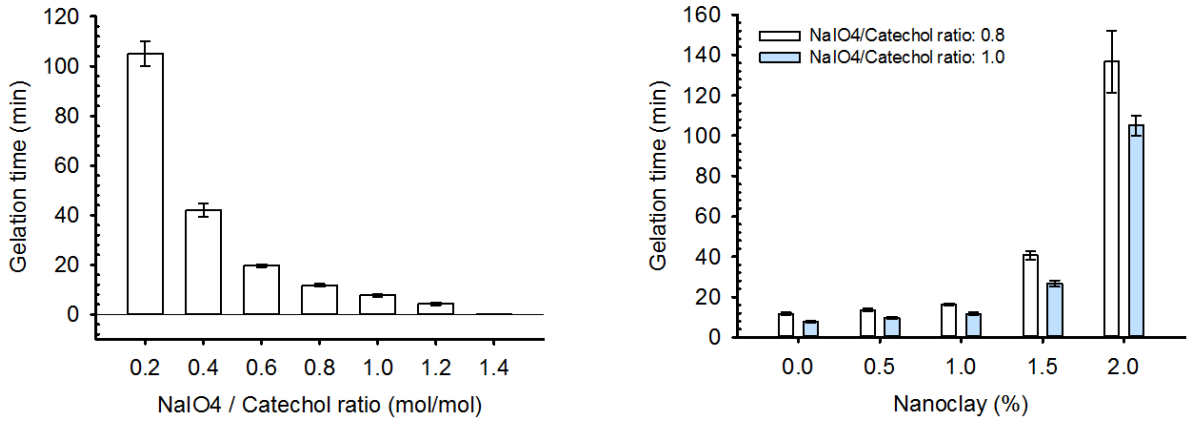


Figure S4. FT-IR spectra of nanoclay, SAG, and SAG-loaded nanoclay.



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44 **Figure S5**. Gelation time of hydrogels at various $NaIO_4$/catechol group ratios and nanoclay

45 contents.

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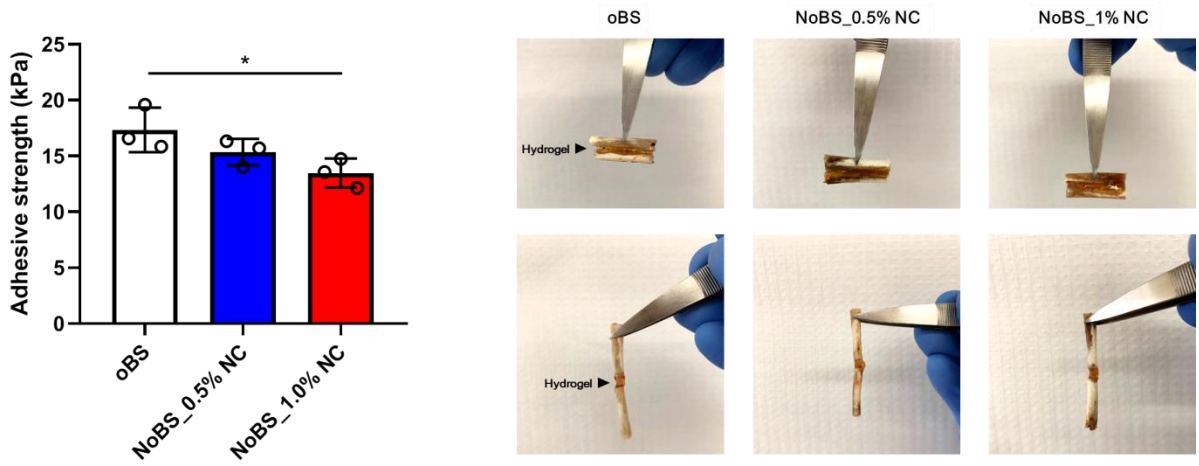

Figure S6. Adhesive strength of NoBS, and photography images of hydrogel adhesiveness to bone. Statistical analysis was determined by one-way ANOVA with Tukey's post hoc test; *$P < 0.05$.

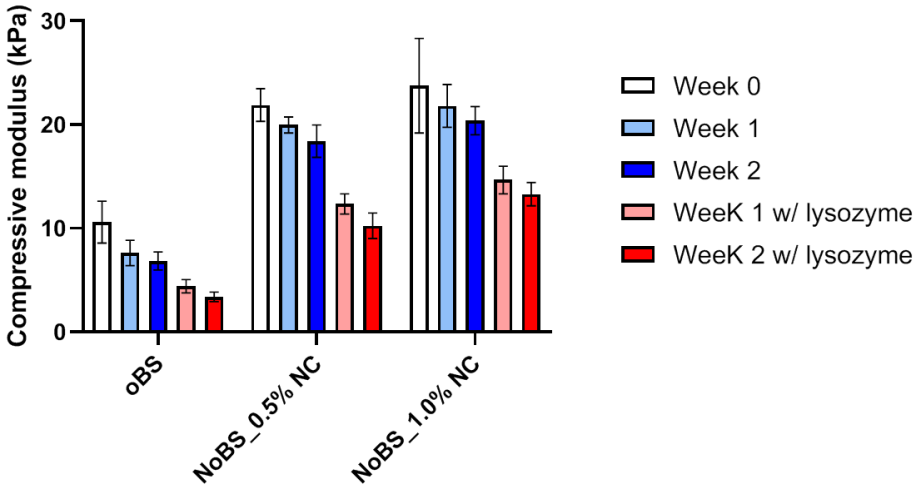

Figure S7. The measurements of compressive modulus for NoBS after degradation for 2 weeks. The hydrogels were incubated in the presence or absence of lysozyme (1 g L^{-1}) to facilitate degradation using lysozyme, a chitosan lytic enzyme.

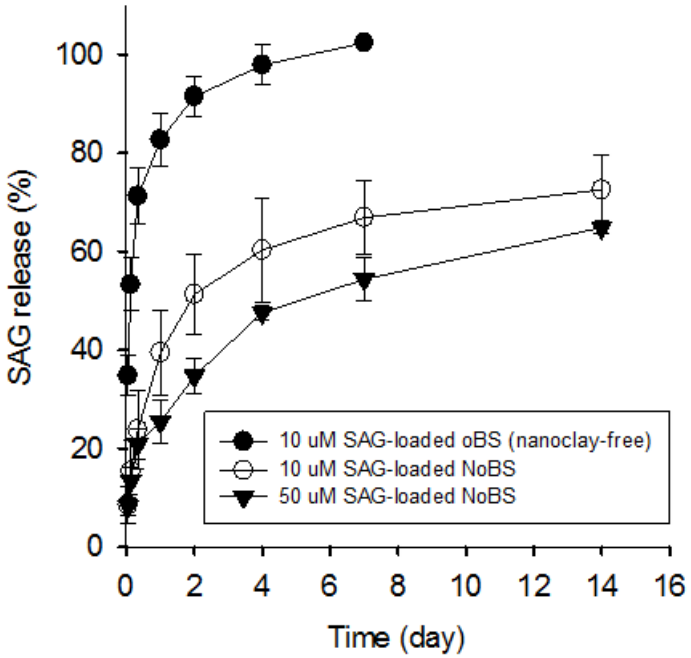

Figure S8. Time-lapsed release profiles of SAG from oBS and NoBSs.

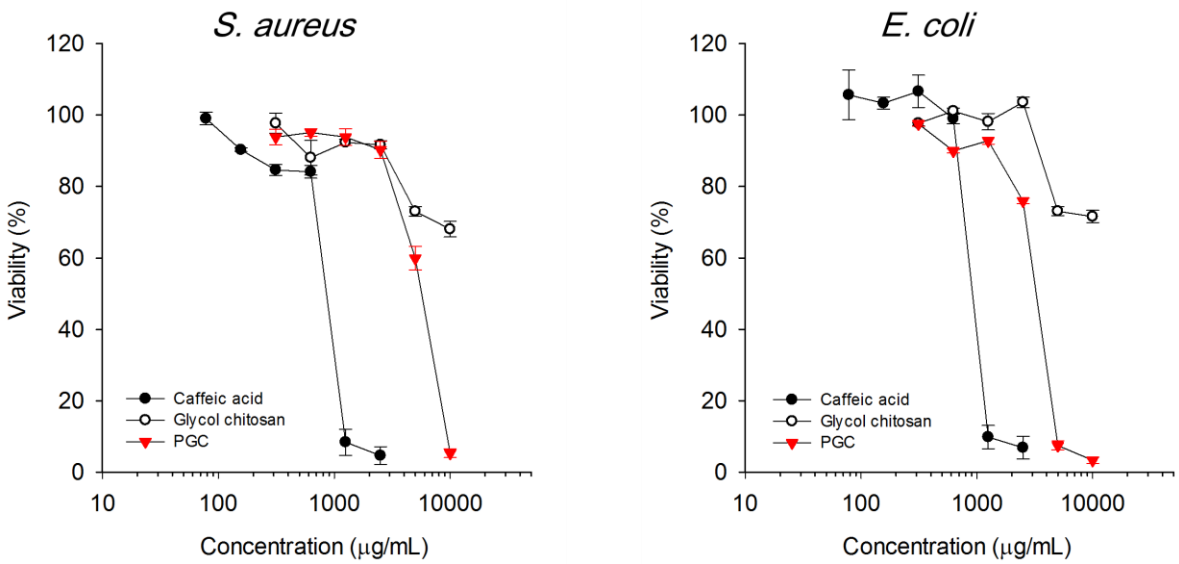

Figure S9. Viability test of caffeic acid, glycol chitosan and the PGC at various concentrations against *S. aureus* and *E. coli* for 24 h.

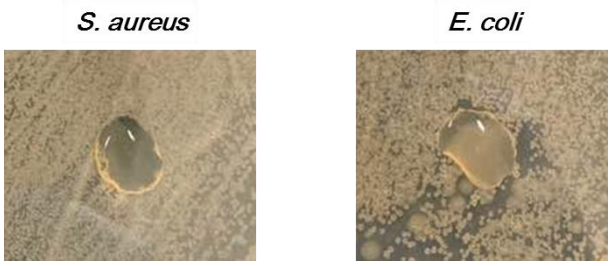

Figure S10. Representative image of bacterial colonies formed by **A)** *S. aureus* and **B)** *E. coli* with methacrylate chitosan hydrogel placed on the agar petri dish for a day.

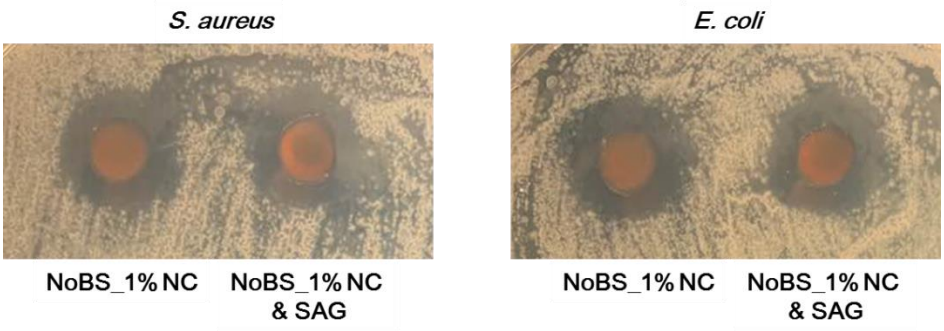

Figure S11. Representative image of bacterial colonies formed by *S. aureus* and *E. coli* with NoBS_1% NC in the presence or absence of SAG placed on the agar petri dish for a day.

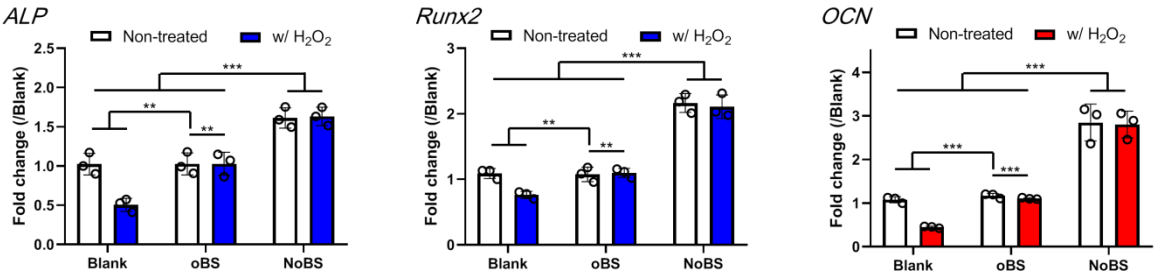

Figure S12. Gene expression related to osteogenesis was evaluated with qRT-PCR with oBS and 1% nanoclay-NoBS in the presence or absence of 0.2 mM H_2O_2. *ALP* and *Runx2* were examined at day 4, and *OCN* was measured at day 14. Error bars indicate standard deviation (three independent cultures, n = 3), $^{**}p < 0.01$, and $^{***}p < 0.001$ (ANOVA followed by Tukey's post hoc test).

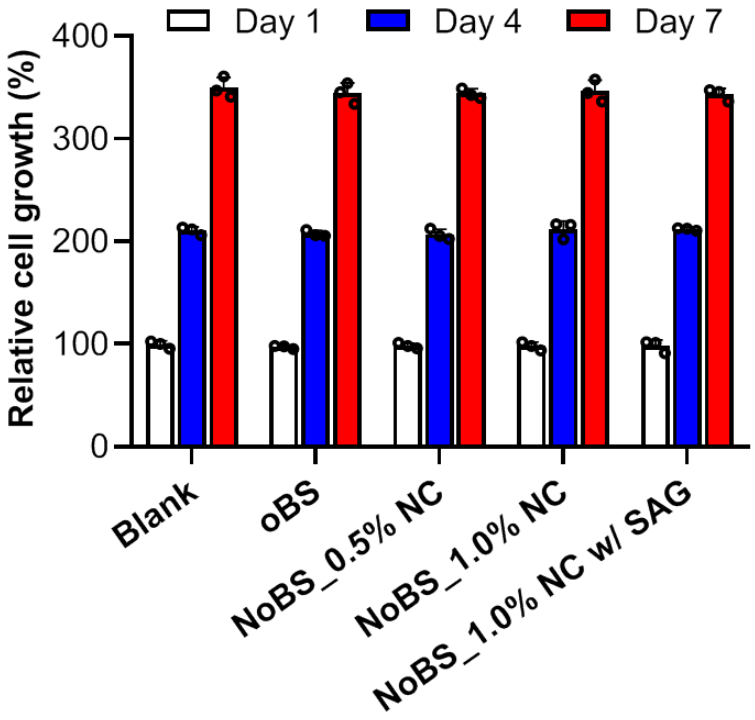

Figure S13. In vitro 2D cell proliferation assay of BMSCs incubated with oBS and NoBSs at various amounts of nanoclay (0.5 and 1.0%) in the presence or absence of 10 μM SAG for 7 days. The value was normalized by blank group of Day 1.

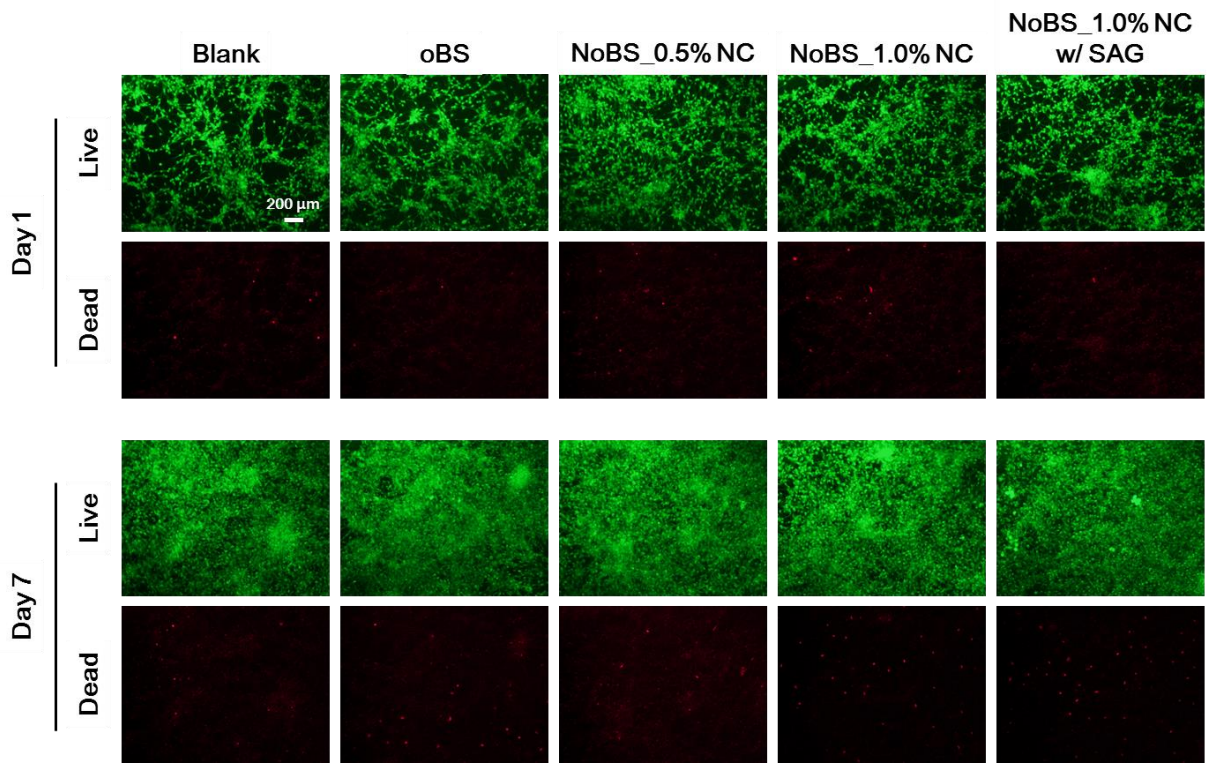

Figure S14. Representative confocal fluorescence images of BMSCs incubated with oBS and NoBSs at various amounts of nanoclay (0.5 and 1.0%) in the presence or absence of 10 μM SAG. The cells were stained with calcein AM (live cells, green fluorescence) and ethidium homodimer (dead cells, red fluorescence) at day 1 and day. Scale bar indicates 200 μm.

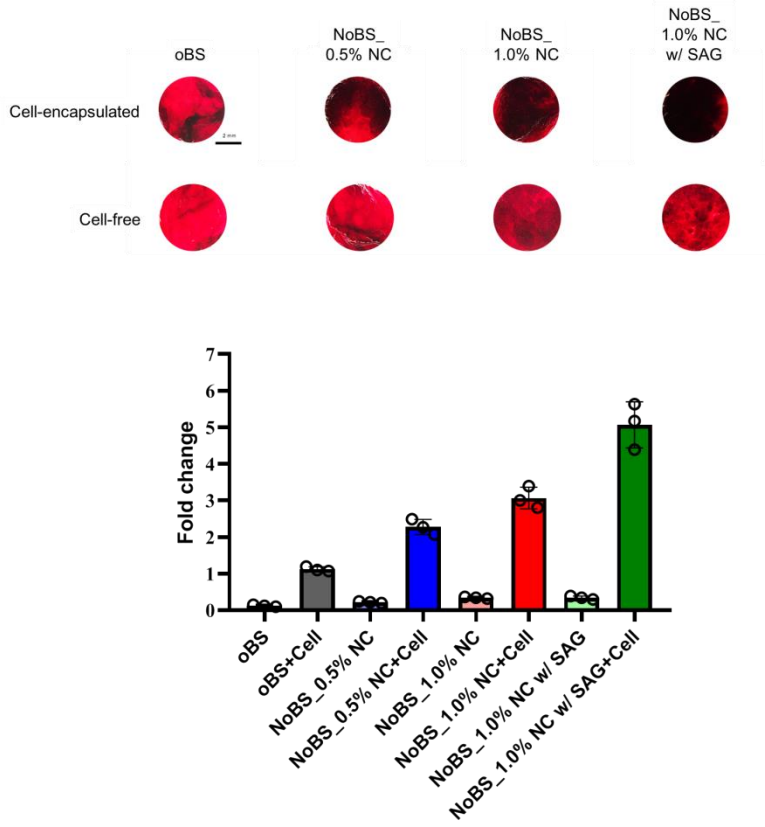

Figure S15. Alizarin red S staining of NoBS hydrogels. Relative colorimetric quantification of alizarin red S staining was compared between cell-encapsulating and cell-free groups. Scar bar indicates 2 mm. The concentration of SAG for SAG-containing groups was 10 μM.

Table S1. Interplanar distances (d_{hkl}) and 2 θ (λ=1.54 Å) of nanoclay and SAG-loaded nanoclay determined by XRD data.

Diffraction plane (*hkl*)	**Nanoclay**		**SAG-loaded nanoclay**	
	***θ* (°)**	***d* (Å)**[1)]	***θ* (°)**	***d* (Å)**
(001)	7.83	11.29	7.65	11.56
(110, 020)	19.72	4.50	19.61	4.53
(004)	27.71	3.22	27.47	3.25
(130, 200)	35.05	2.56	34.73	2.58
(150, 240, 310)	50.78	1.80	50.36	1.81
(060, 330)	60.84	1.52	60.70	1.53

[1)] The *d*-spacing was calculated by Bragg's equation.

Table S2. Elemental composition of the surface for oBS and NoBSs with various amounts of nanoclay (0.5 and 1.0%) in the presence or absence of 10 μM SAG determined by energy dispersive X-ray spectrometry.

Atom %	**oBS**	**NoBS_0.5% NC**	**NoBS_1.0% NC**	**NoBS_1.0% NC w/ SAG**
C	54.57±1.41	48.59±1.59	47.79±1.48	47.19±1.79
N	13.30±4.99	-	-	-
O	32.13±1.59	37.23±1.15	38.33±1.04	38.24±1.25
F	-	1.59±0.51	1.77±0.52	1.99±0.60
Na	-	2.00±0.17	1.72±0.14	1.64±0.20
Mg	-	3.53±0.14	3.48±0.19	3.41±0.23
Si	-	5.89±0.18	5.66±0.22	5.57±0.26
S	-	-	-	0.66±0.11
Cl	-	1.18±0.13	1.25±0.11	1.30±0.13

Table S3. Sequences of primers for qPCR assay.

Primers	Forward	Reverse
GAPDH	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
ALP	GTTGCCAAGCTGGGAAGAACAC	CCCACCCCGCTATTCCAAAC
Runx2	CGGTCTCCTTCCAGGATGGT	GCTTCCGTCAGCGTCAACA
OCN	GGGAGACAACAGGGAGGAAAC	CAGGCTTCCTGCCAGTACCT
GSK-3	GCGTTCCCAAGAAGTGGCTTA	GGTCCAGCTTACGCATAATCTG
β-catenin	ATGGAGCCGGACAGAAAAGC	CTTGCCACTCAGGGAAGGA
PTCH	CAGGCTTCCTGCCAGTACCT	GACAATGATTCCAGCAGTCCAAG
Gli1	ACACATTACCAAGAAGCACCG	CAGCTGGTTTTCCCCTTTAAC