Supporting Information

In Situ Bone Regeneration Enabled by a Biodegradable Hybrid Double-Network Hydrogel

Yuanhao Zhang[#a], Mingjiao Chen[#b], Jia Tian[a], Ping Gu[b], Hongliang Cao[a], Xianqun Fan*[b] and Weian Zhang*[a]

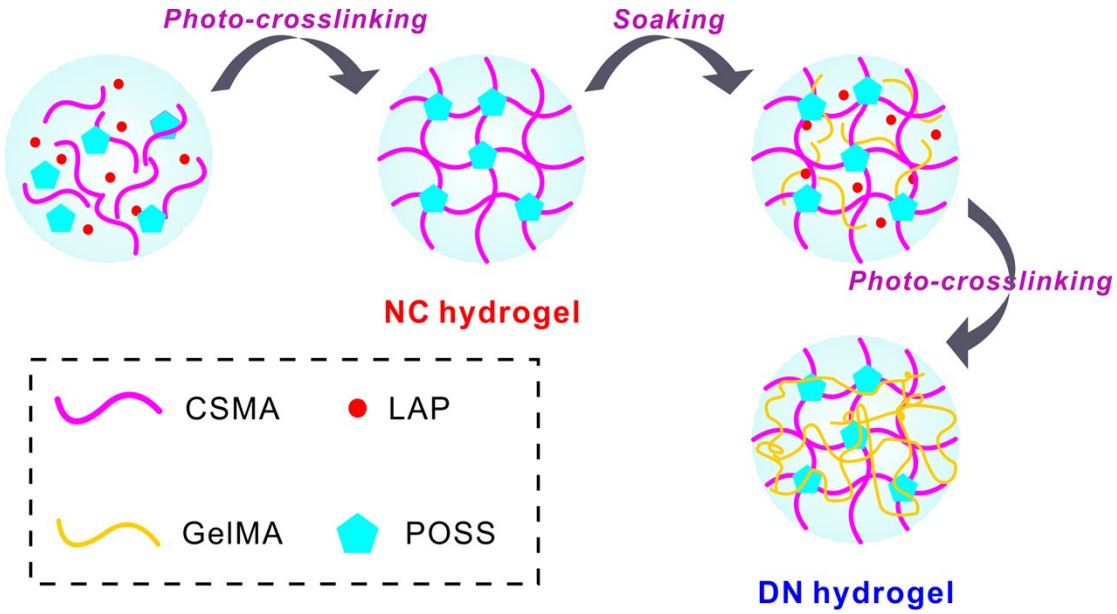

Fig. S1 The biodegradable hybrid DN hydrogel prepared *via* two-step photo-crosslinking process.

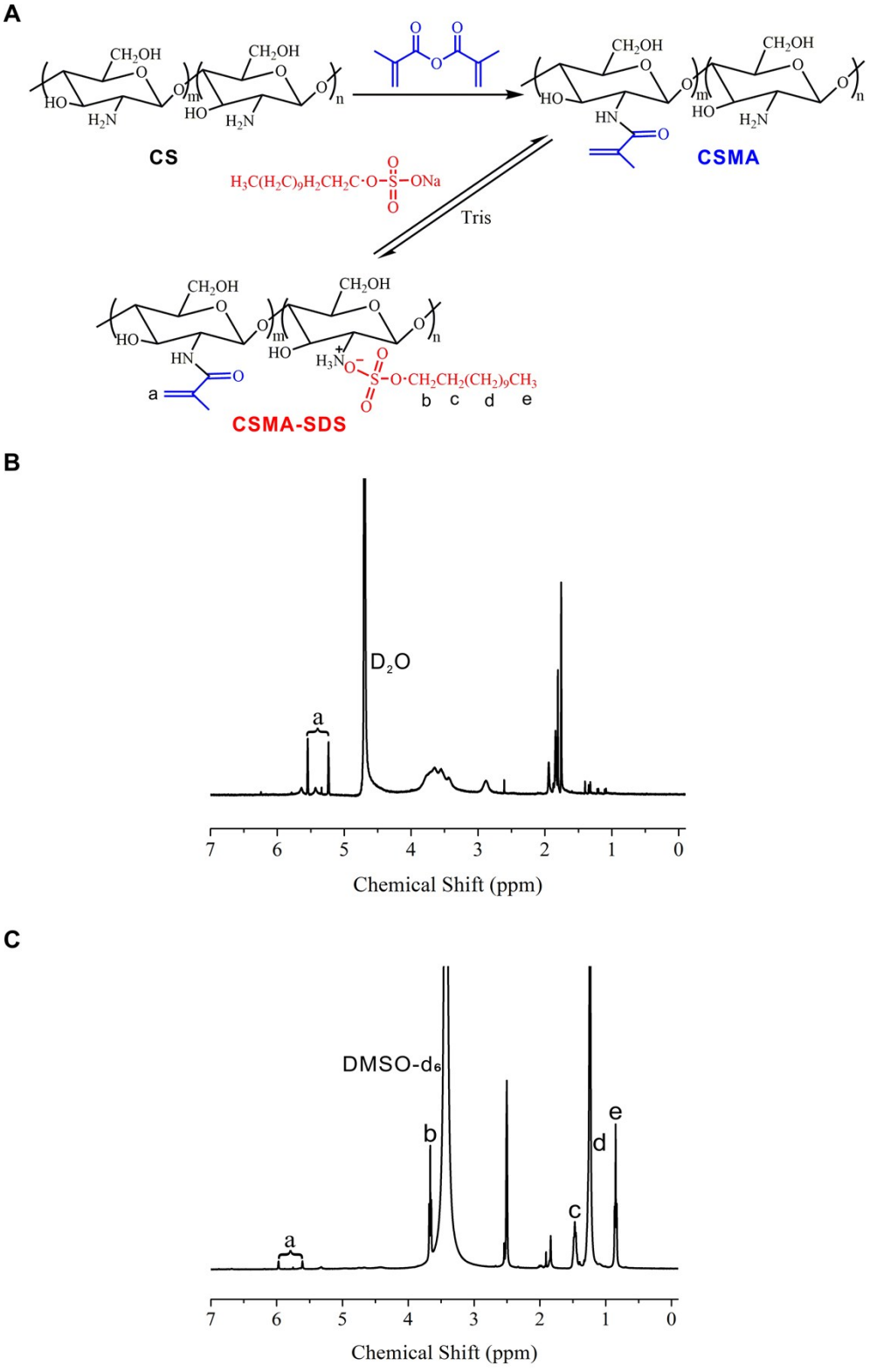

Fig. S2 Preparation and characterization of CSMA and CSMA-SDS. A) Synthesis routine of CSMA and CSMA-SDS; B) ^{1}H-NMR spectrum of CSMA in D_2O. The present of new peaks located at 5.6 and 6.0 ppm attribute to the methylene protons of methacrylate. C) ^{1}H-NMR spectrum of CSMA-SDS in DMSO-d_6. The peaks at 0.86, 1.25, 1.49 and 3.69 ppm are the characteristic alkyl chain peaks of SDS.

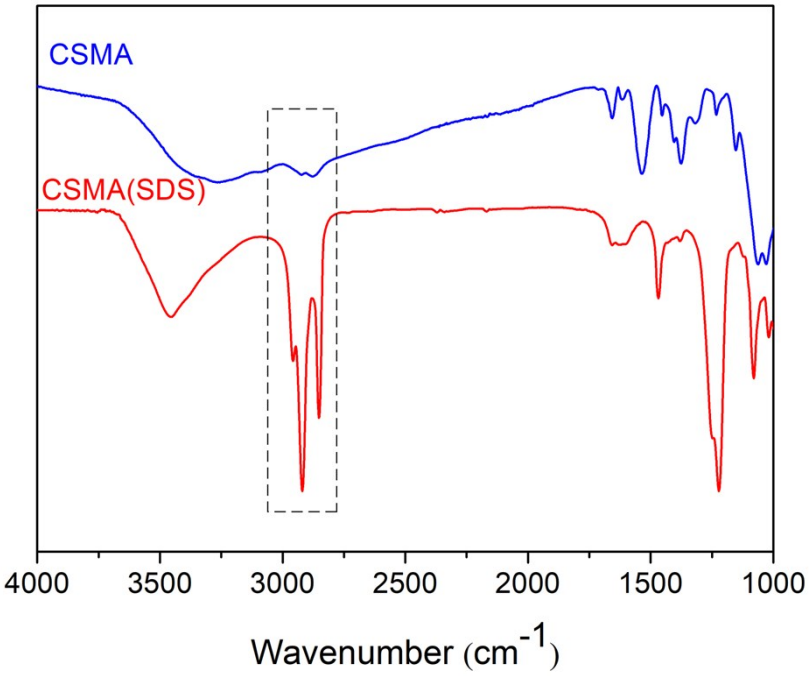

Fig. S3 FT-IR spectra of CSMA and CSMA-SDS. The strengthened intensity of absorption band at 2835-2965 cm^{-1} (dashed box) attributes to the stretch mode of methylene of SDS.

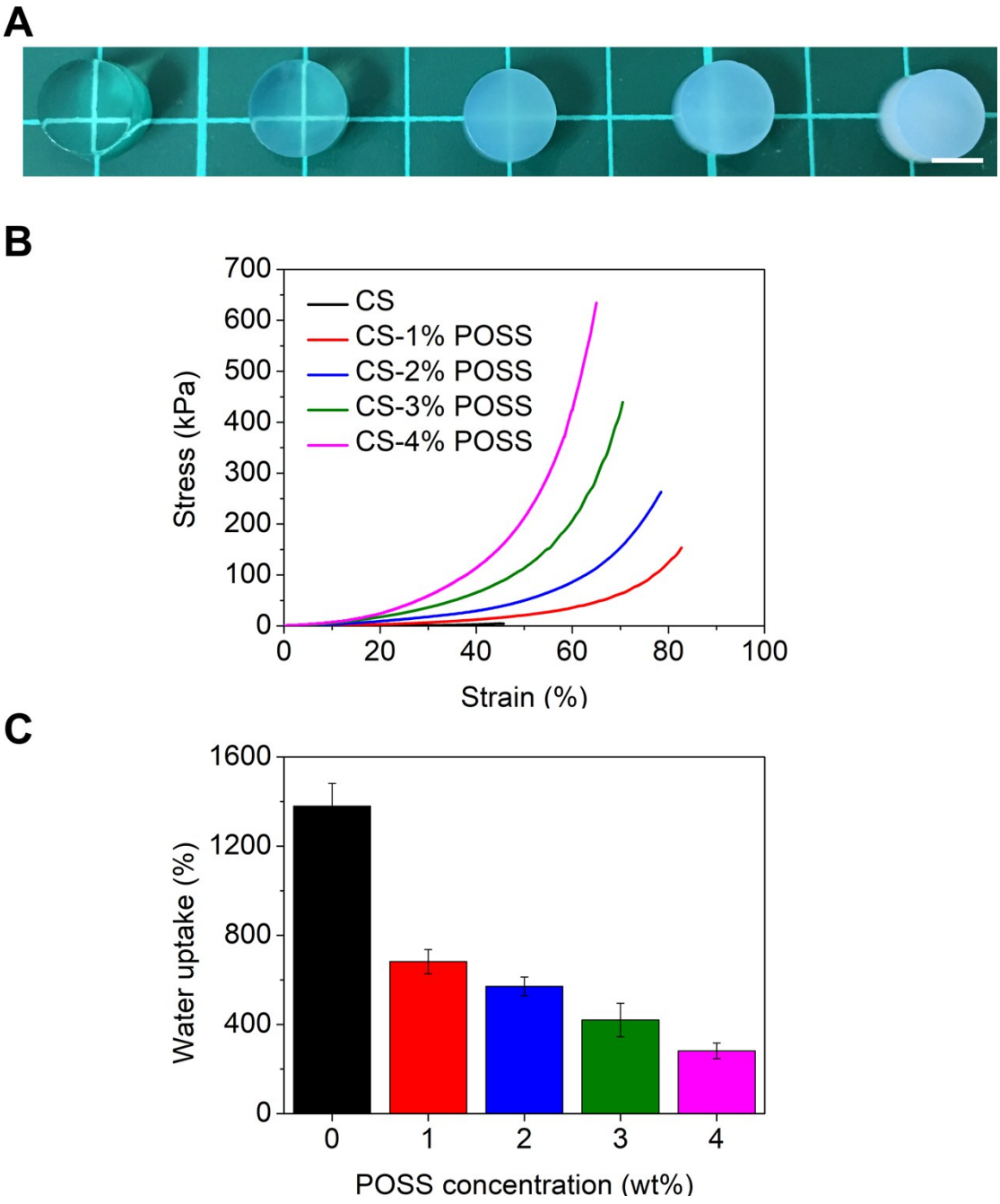

Fig. S4 Characterization of CS-POSS hybrid hydrogels. A) Optical images of hydrogels with different POSS concentration. They are CS, CS-1% POSS, CS-2% POSS, CS-3% POSS and CS-4% POSS from left to right, respectively. The scale bar is 5 mm. B) Compression curves of hydrogels. C) Water uptake (%) of freeze-dried hydrogels in PBS.

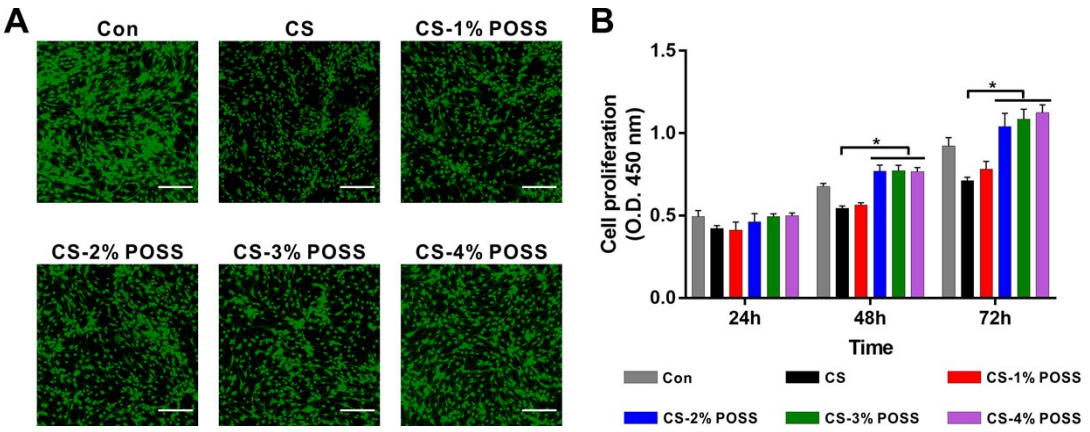

Fig. S5 Cell behaviors on CS-POSS hydrogels. A) Live/dead staining of MSCs for 3 days. Live cells are green and dead cells are red. The scale bar is 500 μm. B) Cell proliferation on hydrogels for 3 days. *$p < 0.05$.

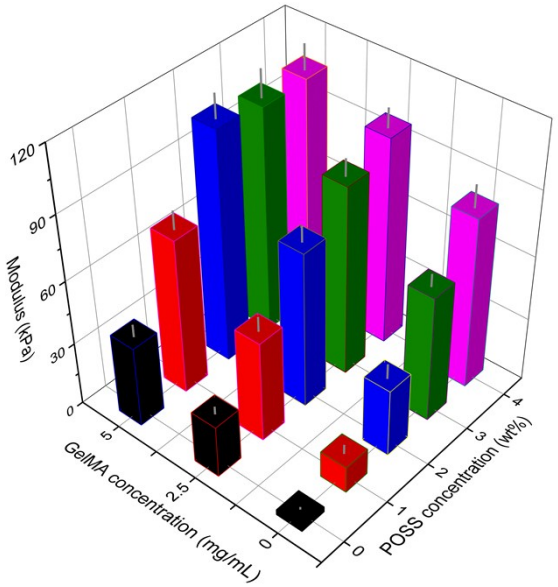

Fig. S6 Compression modulus of DN hydrogels with different POSS and GelMA concentration.

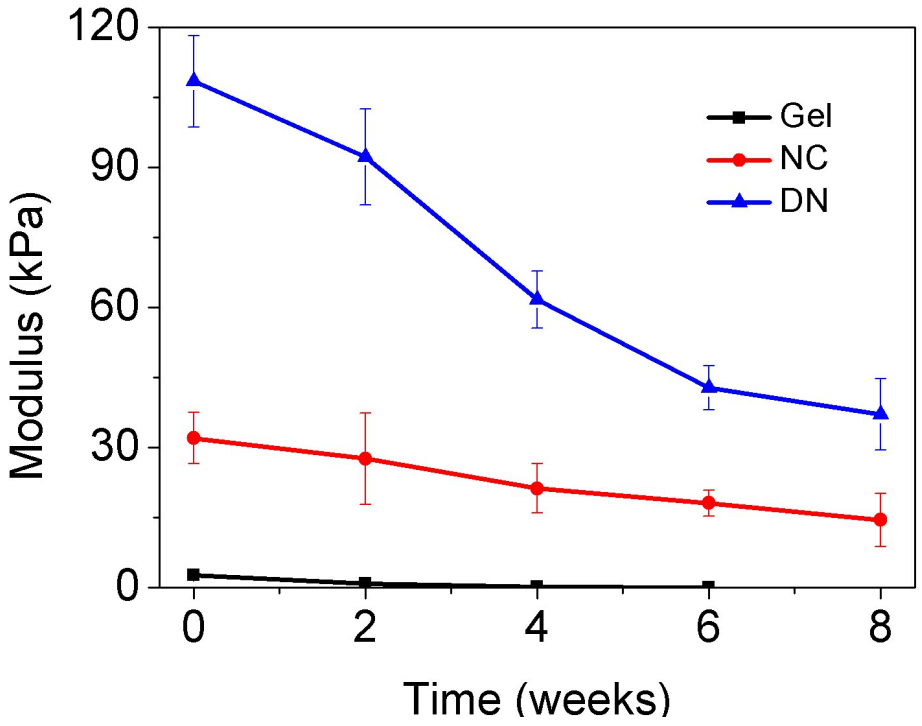

Fig. S7 The decline of modulus of hydrogels in the degradation medium during 8 weeks.

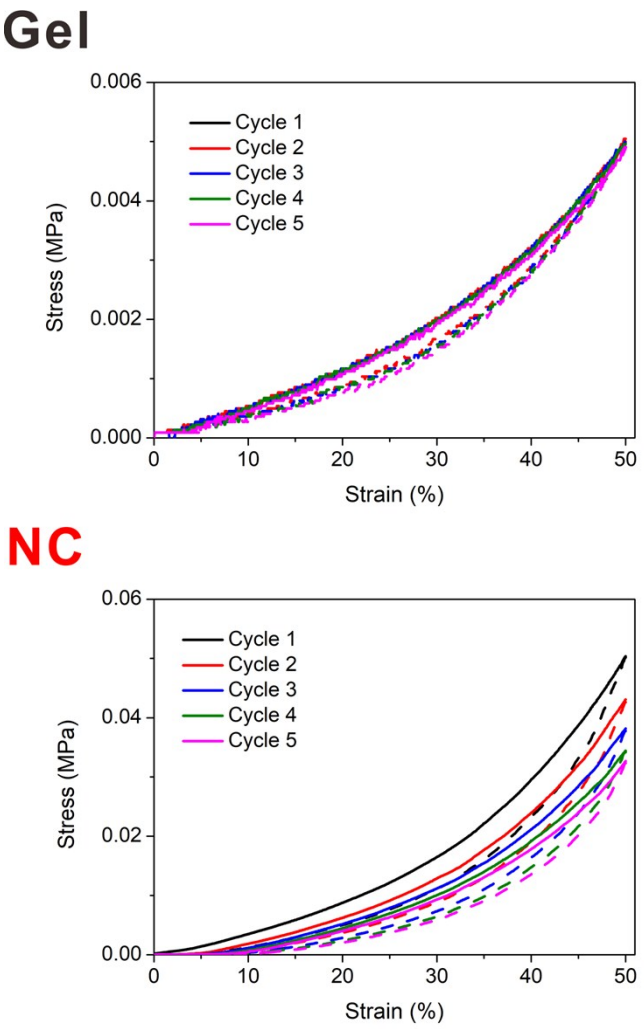

Fig. S8 Stress-strain curves of Gel and NC hydrogels under cyclic compression with a peak strain of 50%.

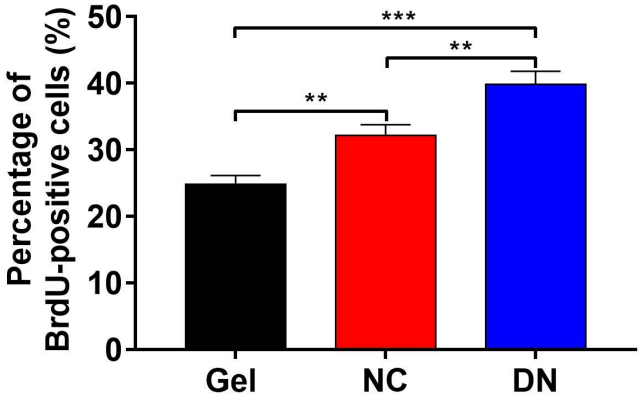

Fig. S9 The percentage of BrdU-positive cells. *p < 0.05, **p < 0.01 and ***p < 0.001.

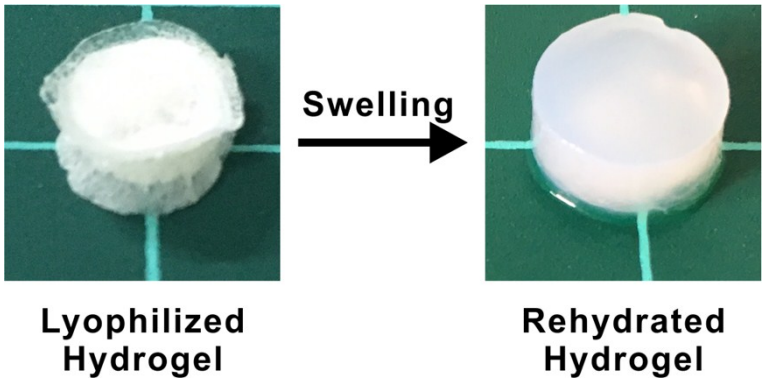

Fig. S10 Images of lyophilized and rehydrated DN hydrogel.

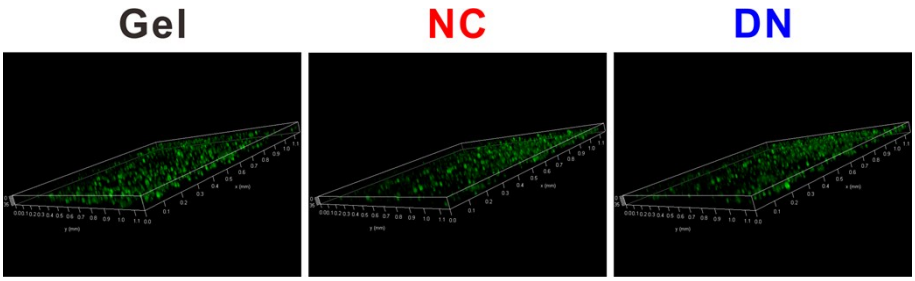

Fig. S11 The cell viability in hydrogels after incubation of 24 h.

Table S1 Summary of the physical properties of hydrogels.

Properties	Gel hydrogel	NC hydrogel	DN hydrogel
Porosity (%)	91 ± 5	87 ± 2	76 ± 4
Water content (%)[a]	92.3/85.7	85.1/79.5	89.8/87.5
Modulus (kPa)[b]	2.6 ± 0.6	32.0 ± 5.5	108.5 ± 9.8

[a] water content ratio before and after centrifugation. [b] measured in compression.

Table S2 Primers and parameters used for the qPCR analysis

Gene Name	Accession No.	Primer sequence	Annealing temperature (℃)	Product size (base pairs)
BSP	NM_004967	Forward:5'-CACTGGAGCCAATGCAGAAGA-3' Reverse:5'-TGGTGGGGTTGTAGGTTCAAA-3'	60	106
Runx2	NM_001015	Forward:5'-TGGTTACTGTCATGGCGGGTA-3' Reverse:5'-TGGTTACTGTCATGGCGGGTA -3'	60	101
OSX	NM_152860	Forward:5'-CCTCTGCGGGACTCAACAAC-3' Reverse:5'-AGCCCATTAGTGCTTGTAAAGG-3'	60	128
OPN	NM_001251	Forward:5'-CTCCATTGACTCGAACGACTC-3' Reverse:5'-CAGGTCTGCGAAACTTCTTAGAT-3'	60	230
Col1A1	NM_000088	Forward:5'-GAGGGCCAAGACGAAGACATC-3' Reverse: 5'-CAGATCACGTCATCGCACAAC-3'	60	140
GAPDH	NM_001256	Forward:5'-GGAGCGAGATCCCTCCAAAAT-3' Reverse:5'-GGCTGTTGTCATACTTCTCATGG-3'	60	197