Supplementary file

2. Materials and methods

2.4.1. Morphological characterization

The microstructure of the NAGA, NGL3, NGL5, T5, T10 and TA samples were observed using scanning electron microscopy (SEM; Merlin, Zeiss, Germany).

2.4.2. FTIR analysis

FTIR spectrometry spectroscopy (FTIR, Nicolet iN10, Thermo Scientific, USA) was used to identify the chemical structure of the samples of LPN, NAGA, GelMA, and NGL as well as T5 hybrid hydrogel, respectively. FTIR spectra with a wavenumber range of 675 to 4000 cm^{-1} and an average of 32 scans.

2.4.3. Swelling test

The dynamic swelling behavior of the hydrogel was measured. The crosslinked cylinder samples (n=3) with a diameter of 10 mm and a height of 5 mm were immersed in phosphate-buffered saline (PBS) at 37 °C for 48 h. Before weighed, the surface water of the hydrogel was removed gently with a filter paper, and the swollen hydrogel was weighed. The swelling ratio could be calculated using the following formula:

$$\text{Swelling ratio}=\frac{W_s-W_d}{W_d}\times 100\%.$$

where Wd is the dry weight of the hydrogel, and Ws is the swollen weight of the hydrogel at any given time.

2.6.1. Assays on cytocompatibility

The hydrogels were exposed to ultraviolet light overnight for the cell proliferation assay, followed by rinsing with phosphate-buffered saline (PBS) and a culture medium. Bone marrow mesenchymal stem cells (BMSCs) were kindly provided by Stem Cell Bank, Chinese Academy of Sciences, and expanded in proliferation medium consisting α-MEM, 10% fetal bovine serum (FBS) and 1% penicillin−streptomycin (PS,Gibco, USA). BMSCs were cultured at a density of 1×10^3 cells/well in a 96-well plate for 1, 3, and 7 days. Subsequently, cell counting kit-8 (CCK8) solution (10%, Dojindo, Japan) was added to each well, and the optical density (OD) values (450 nm) were collected to analyze the cell proliferation. The cell proliferation could be calculated using the following formula:

$$\text{Cell proliferation (\%)} = \frac{OD_s - OD_b}{OD_c - OD_b} \times 100\%.$$

where ODs is the OD values of the sample, ODb is the OD values of the blank group, and ODc is the OD values of the control group.

2.6.2. Assays on osteogenic and chondrogenic in cell culture

To evaluate osteogenic differentiation on the hydrogels, alizarin red staining (ARS), alkaline phosphatase (ALP) staining, and quantitative real-time polymerase chain reaction (qRT-PCR) assays were performed. For ARS and ALP, 2×10^4 BMSCs were seeded onto the hydrogels and cultured in osteogenic differentiation medium. For the ALP staining, BMSCs were cultured for 7 and 14 days in two 24-well culture plates. The hydrogels and culture medium were removed, and the cells were fixed and incubated with ALP dye liquor (Beyotime) according to the manufacturer's instructions for 1 h. For the ARS, BMSCs were cultured for 21 days in one 24-well culture plate,

incubated with 2% alizarin red (Cyagen, China) at pH 4.2 for 10 min, and then washed with distilled water. Light microscopy was used to observe the ALP and ARS. A qRT-PCR was also performed to analyze the osteogenic differentiation of the BMSCs. BMSCs (2×10^4) were seeded onto each hydrogel and cultured for 14 days. RNA was extracted using the TRIzol reagent (Invitrogen). Complementary DNA was generated using a RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific), and the qRT-PCR was performed using an ABI Prism 7300 thermal cycler (Applied Biosystems). GAPDH gene was used as a control to normalize the gene expression. The expression levels of GAPDH, collagen type I (Col I), runt-related transcription factor 2 (RUNX2), osteocalcin (OCN), and ALP were measured. All the samples were measured in triplicate, and the relative gene expression was calculated using the formula $2^{-\Delta\Delta CT}$.

To evaluate chondrogenic differentiation on the hydrogels, qRT-PCR assays were performed. The BMSCs planted on hydrogels for 14 days were washed with PBS, collected in EP tubes with RNA later solution (Ambion), and stored at −20 ℃. The RNA of the experimental sample was extracted using the RNeasy Mini Kit (Qiagen), and the concentration of RNA was detected using a microspectrophotometer (ND1000, Nanodrop Technologies). Then an iScriptTM cDNA Synthesis Kit (BIO-RAD) was used to reverse transcript (RT) the extracted RNA to cDNA, and SoFastTM EvaGreen Supermix (BIO-RAD) was used to conduct a polymerase chain reaction (PCR) with a CFX96 TouchTM Real-Time PCR Detection System (BIO-RAD). The expression levels of GAPDH, SOX9, collagen type II (Col II), Collagen Type X (Col X), and Aggrecan (AGG) were tested. All the samples were measured in triplicate, and the relative gene expression was calculated with the formula $2^{-\Delta\Delta CT}$.

3. Results

Table S1. Compressive test results for PNAGA -30, PNAGA 30%-Clay, PNAGA30% +NAGA4%, PVA/PNAGA-10/30, P(NAGA-VPA)/HAp, PN-30/PB, PNIPAm/ PNAGA-4, T5 hydrogel

Sample	**Compressive strength (MPa)**	**Compression modulus (MPa)**	**Ref**
PNAGA -30	3.623 ± 0.658	0.322 ± 0.057	1
PNAGA 30%-Clay	3.466±0.674	0.317±0.016	2
PNAGA30%+NAGA4%	4.6		3
PVA/PNAGA-10/30	4	-	4
P(NAGA-VPA)/HAp	3.527 ± 0.269	-	5
PN-30/PB	0.2	-	6
PNIPAm/PNAGA-4	1	-	7
T5	5.467±0.346	0.654±0.063	This work

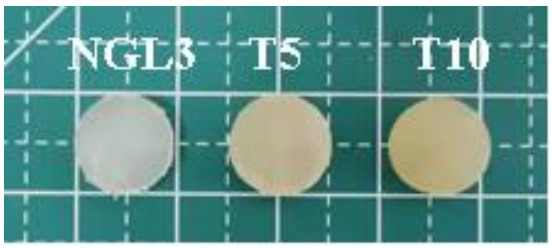

Figure S1. Photographs of NGL3, T5 and T10 hydrogel

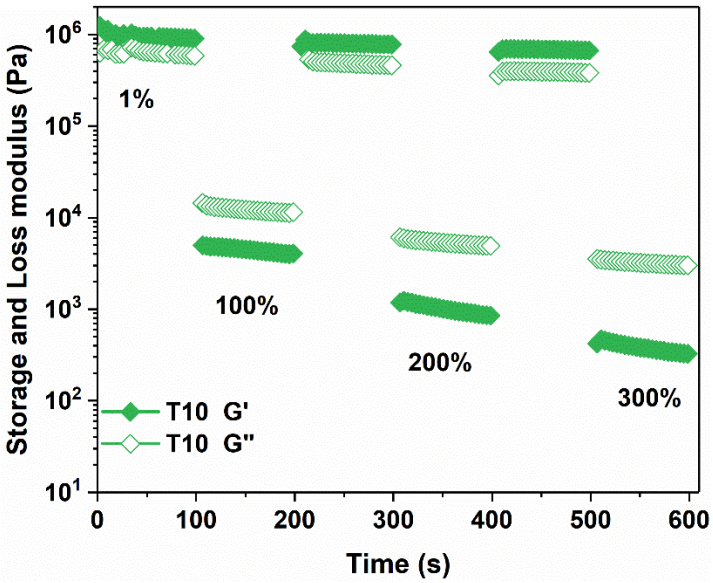

Figure S2. The variation in the G' and G" values of T10 hydrogel results when switching the alternate step strain from minor strain (1%) to enormous strain (100%, 200%, and 300%) at a fixed frequency of 1 Hz and temperature of 25 ℃.

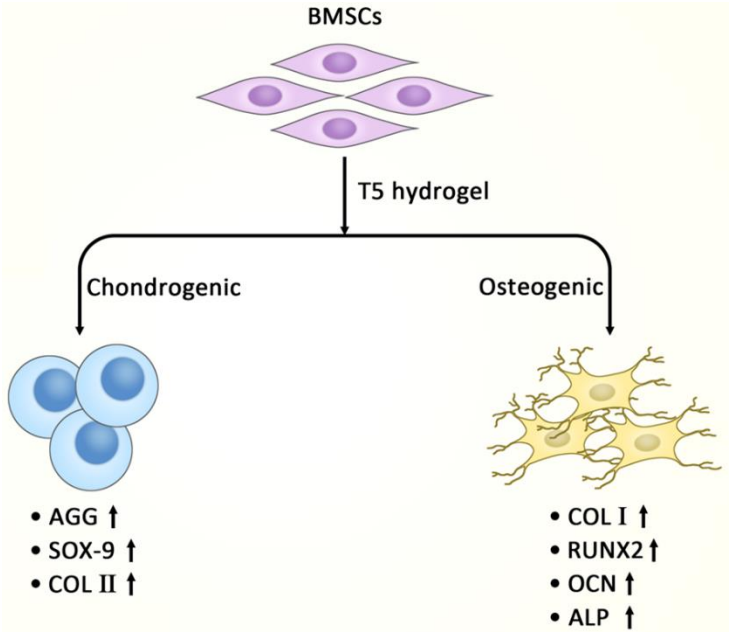

Figure S3. The chondrogenic and osteogenic differentiation of BMSCs under the action of T5 hydrogel.

Reference

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