Electronic Supplementary Information

Low Molecular Weight Gels Induced Differentiations of Mesenchymal Stem Cells

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Experimental section

Synthesis of compound 1: A mixture of 3-aminobenzeneboronicm acid (2.74 g, 20 mmol), 2,2-dimethyl-1,3-propanediol (2.29 g, 22 mmol), and chloroform (40 mL) was stirred magnetically at room temperature for 5 h. The mixture was washed with water. The organic phase was separated, dried over anhydrous sodium sulfate, and filtered. The solvent was evaporated under vacuum, the residue was reprecipitated in isopropanol to obtain compound 1 (yield: 3.89g, 95%). White solid, mp: 108.8-109.4 °C. ^{1}H NMR (500 MHz, $CDCl_3$, TMS), δ1.01 (s, 6H), δ 3.50 (br, 2H), δ 3.75 (s, 4H), δ 6.74-6.77 (m, 1H), δ 7.13-7.25 (m, 3H). ^{13}C NMR (125MHz, $CDCl_3$, TMS), δ (21.9, 31.9, 72.3, 117.6, 120.4, 124.2, 128.6, 145.7). HRMS (ESI^+) calcd for $(C_{11}H_{17}BNO_2)^+$: 206.1341; found: 206.1349.

Synthesis of compound 2 (Boc-Gly-Gly-OH): 1,4-dioxane (35 mL) and Et_3N (11 mL) were added to a solution of H-Gly-Gly-OH (6.61g, 50 mmol) in deionized water (35 mL) , followed by Boc_2O (12 g, 12.5 mL, 55 mmol). The solution was stirred for 16 h at room temperature, followed by treatment with a solution of deionized water (25 mL) and EtOAc (120 mL). The aqueous phase was washed with EtOAc (120 mL) again, followed by acidification with citric acid solids to pH 2.5. The aqueous phase was extracted with EtOAc (120 mL) twice. The EtOAc phase was dried over anhydrous sodium sulfate, and evaporated under vacuum to obtain a white solid (yield: 8.5g, 67%). White solid, mp: 122.5-122.7 °C. ^{1}HNMR (500 MHz, $CDCl_3$, TMS) δ 1.38 (s, 9H), δ 3.56 (d, 2H, J = 6.0 Hz), δ 3.76 (d, 2H, J = 6.0 Hz), δ 7.01(t, 1H, J = 6.0 Hz), δ 8.07 (t, 1H, J = 6.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$, TMS) δ (28.2, 40.5, 43.0, 78.0, 155.7, 169.7, 171.2).

Synthesis of compound 3: 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetra-methyluronium tetrafluoroborate (TBTU) (3.211 g, 10 mmol) was added to a mixture of compound 2 (2.320 g, 10 mmol), triethylamine (2.8 mL, 20 mmol) in 40 mL ethyl acetate, then stirred at room temperature for 10 min. Afterward compound 1 (2.46 g, 12 mmol) was added to the solution and stirred at room temperature for 24 h. Then the mixture was filtered, and the solid was reprecipitated in dichloromethane/ethanol to obtain compound 3 (yield: 2.43 g, 58%). White solid, mp: 187.5-187.8 °C. ^{1}HNMR (500 MHz, DMSO, TMS) δ 0.96 (s, 6H), δ 1.40 (s, 9H), δ 3.61 (d, 2H, J=6.0 Hz), δ 3.75 (s, 4H), δ 3.88 (d, 2H, J=6.0 Hz), δ 7.11 (t, 1H, J=6.0Hz), δ 7.29(t, 1H, J=7.5Hz), δ 7.38 (d, 1H, J=7.5Hz), δ 7.76 (d, 1H, J=8.0Hz), δ 7.90 (s, 1H), δ 8.18 (t, 1H, J=6.0Hz), δ 9.72 (s, 1H). ^{13}C NMR (125 MHz, DMSO, TMS) δ (21.3, 28.3, 31.4, 42.7, 43.4, 71.3, 78.2, 121.6, 124.6, 128.0, 128.5, 138.1, 156.0, 167.5, 170.0).

Synthesis of compound 4: Compound 3 (4.0 g, 9.5 mmol) was dissolved in CH_2Cl_2 (7 mL). TFA (3.5 mL) was added to the solution and stirred for 4 h at room temperature. The solution was

evaporated under vacuum to remove CH_2Cl_2 and TFA. Then acetonitrile was added, and the mixture was filter. The solid was reprecipitated in ethyl acetate to obtain compound 4 (yield: 2.48 g, 82%). White solid, mp: 192.9-193.8 °C. ^{1}HNMR (500 MHz, DMSO-d_6, TMS) δ 0.96 (s, 6H), δ 3.67 (s, 2H), δ 3.75 (s, 4H), δ 3.99 (d, 2H, J=5.5Hz), δ 7.29 (t, 1H, J=7.5Hz), δ 7.39 (d, 1H, J=7.5Hz), δ 7.72 (d, 1H, J=8.0Hz), δ 7.89 (s, 1H), δ 8.11 (s, 2H), δ 8.75 (t, 1H, J=5.5Hz), δ 10.01 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ (21.2, 31.4, 42.7, 71.4, 121.7, 124.6, 128.0, 128.6, 138.1, 166.5, 167.0).

Synthesis of compound 5: Glycine methyl ester hydrochloride (2.51 g, 20 mmol) was dissolved in dried CH_2Cl_2 and cooled in ice bath. The desired acyl chloride (24 mmol) was added dropwise to the solution firstly, then triethylamine (6.7 mL, 48 mmol) was added and stirred for 12 h. The reaction mixture was filtered, and the filtrate was washed with deionized water several times. The organic phase was separated, dried over anhydrous sodium sulfate, filtered, and the solvent was evaporated under vacuum to obtain compound 5 (crude product).

Synthesis of compound 6: Compound 5 (10 mmol) was dissolved in the mixture of MeOH and THF ($v_{MeOH: THF}$=5:1, 48 mL). NaOH (0.96 g, 24 mmol) in water (12 mL) was added to the solution at 0 ^{o}C and stirred for 5 h. The solvent was evaporated under vacuum. The residue was cooled in ice bath, and 2M HCl (20 mL) was added dropwise. The white precipitate was extracted with ethyl acetate (150 mL) for three times. The residue was reprecipitated in acetate for three times to obtain compound 6 (yeild: 95%). White solid, mp : 120.6-121.4 °C. ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ 0.86 (t, 3 H, J = 6.75 Hz), δ 1.18-1.30 (m, 16H), δ 1.48 (m, 2H), δ 2.10 (t, 2H, J = 7.5 Hz), δ 3.71(s, 2H), δ 8.08 (s, 1H), δ 12.46 (s, 1H). ^{13}CNMR (125 MHz, DMSO-d_6, TMS). δ (13.89, 22.06, 25.41, 28.58, 28.68, 28.78, 28.92, 28.98, 29.00, 31.27, 35.04, 40.48, 171.37, 172.50).

Synthesis of compound 7: TBTU (1.61 g, 5 mmol) was added to a mixture of compound 6 (5 mmol) and triethylamine (1.4 mL , 10 mmol) in 30 mL ethyl acetate, and stirred at room temperature for 10 min. Afterward compound 4 (1.23 g, 6 mmol) was added to the solution and stirred at room temperature for 36 h . After completion of the reaction, the mixture was filtered. The solid was reprecipitated in ethanol to obtain compound 7 (yield, 55%). White solid, mp: 203.1-204.4 °C. ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ 0.85 (t, 3 H, J=5 Hz), δ 0.95 (s, 6H), δ 1.16-1.31 (m, 16H), δ 1.41-1.53 (m, 2H), δ 2.12 (t, 2H, J=5 Hz), δ 3.72 (d, 2H, J=5 Hz), δ 3.75 (s, 4H), δ 3.77 (d, 2H, J=5 Hz), δ 3.87 (d, 2H, J=10 Hz), δ 7.28 (dd, 2H, J=5, 10Hz), δ 7.38 (d, 1H, J=10 Hz), δ 7.70 (d, 1H, J=10 Hz), δ 7.94 (s, 1H), δ 8.09 (t, 1H, J=5 Hz), δ 8.21 (t, 2H, J=5 Hz), δ 9.71 (s, 1H). ^{13}CNMR (125 MHz, DMSO-d_6, TMS) δ (13.80, 18.50, 21.26, 22.04, 25.03, 28.67, 28.77, 28.88, 28.95, 28.99, 31.25, 31.40, 35.14, 42.19, 42.70, 56.00, 71.38, 121.68, 124.60, 127.89, 128.55, 138.11, 167.39, 169.23, 169.79, 172.81).

Synthesis of compound 8: compound 7（2 mmol）was added to 1M HCl solution (100 mL), and stirred for 24 h at room temperature. Then the solid was filtered and evaporated under vacuum to obtain compound 8 (yeild: 64%). White solid, mp 249.1-250.8 °C. ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ 0.85 (t, 3H, J = 5 Hz), δ 1.12-1.34 (m, 16H), δ 1.40-1.54 (m, 2H), δ 2.12 (t, 2H, J = 5 Hz), δ 3.36 (s, 1H), δ 3.72 (d, 2H, J = 5 Hz), δ 3.76 (d, 2H, J = 5 Hz), δ 3.88 (d, 2H, J = 10 Hz), δ 7.26

(dd, 1H, J = 5, 10 Hz), δ 7.49 (d, 1H, J = 10 Hz), δ 7.71 (d, 1H, J = 10 Hz), δ 7.86 (s, 1H),δ 8.02 (s, 1H), δ 8.10-8.13 (m, 1H), δ 8.17-8.26 (m, 2H), δ 9.72 (s, 1H). ^{13}CNMR (125 MHz, DMSO-d_6, TMS) δ (14.38, 19.00, 22.53, 25.54, 29.15, 29.16, 29.26, 29.37, 29.44, 29.48, 31.74, 35.65, 42.66, 43.15, 56.50, 121.80, 125.85, 128.05, 129.64, 138.34, 167.82, 169.68, 170.22, 173.28). FT-IR (KBr): 3307, 3073, 2921, 2855, 1652, 1548, 1368, 1127, 1030, 797, 702, 574 cm^{-1}. HRMS (ESI$^+$) calcd for $(C_{24}H_{40}BN_4O_6)^+$: 491.3036; found: 491.3040.

Gel preparation

A weighed amount of the compound 8 in solvents (1.0 mL) was placed in a closed glass vial and heated until complete dissolution of the solid, and transferred to glass bottom cell culture dish. When cooled to room temperature, the gel was obtained. The gelation was simply confirmed by turning the glass vial upside down. The gel prepared in isopropanol was dried by oil pump at room temperature, the gel prepared in PEG200:H_2O=4:1 were freeze-dried.

MSC proliferation and morphology

As previous described, MSCs were harvested from the bone marrow of 10-day-old rat (rattus norregicus) and all experiments were performed within passage 3. Suspension of MSCs at a density of 2×10^4 per well was seeded on the surfaces of gels and incubated for 3, 5 and 7 days, respectively. Cell counting kit-8 (CCK-8) assay was performed to evaluate the proliferations of MSCs on the samples. The proliferation of MSCs was determined by the OD value at 450 nm. Confocal microscopy (CLSM, Olympus IX 95) was used to analyze the cell morphology on the surface after incubated for 3 days. Before observation, the alive cells were stained by fluorescein diacetate (FDA).

Enzyme-linked immunosorbent assay

The runt-related transcription factor-2 (Runx2), alkaline phosphatase (ALP) activity, collagen type I (Col I) and osteocalcin (OCN), Sry related HMG box 9 (Sox9), collagen type II (Col II), Aggrecan (AGG), collagen type X (ColX), were determined using the quantitative enzyme-linked immunosorbent assay (ELISA). After the MSCs were cultured on the samples for 3, 5, 7 and 14 days in the normal culture media (1 ml of α-MEM supplemented with 10% fetal bovine serum and 1% antibiotics), the samples were washed with PBS and 10 μL of Triton X-100 was added to each well. After the MSCs on the samples were frozen and then thawed repeatedly for 3 times, the solution was collected to measure the expressions of Runx2, ALP and Sox9. The cell culture supernatants were collected to measure the Col I, OCN, Col II, AGG and Col X secretions. All the experiments were conducted strictly according to the instructions of the ELISA kits (BlueGene, Bluegene Ltd., Shanghai, China). The expressions were determined by the absorbance measurements performed with a spectrophotometer (MK3, Thermo Electron Ltd., USA) at 450 nm, by comparing the measured OD values to the standard curve plotted using a set of standard samples.

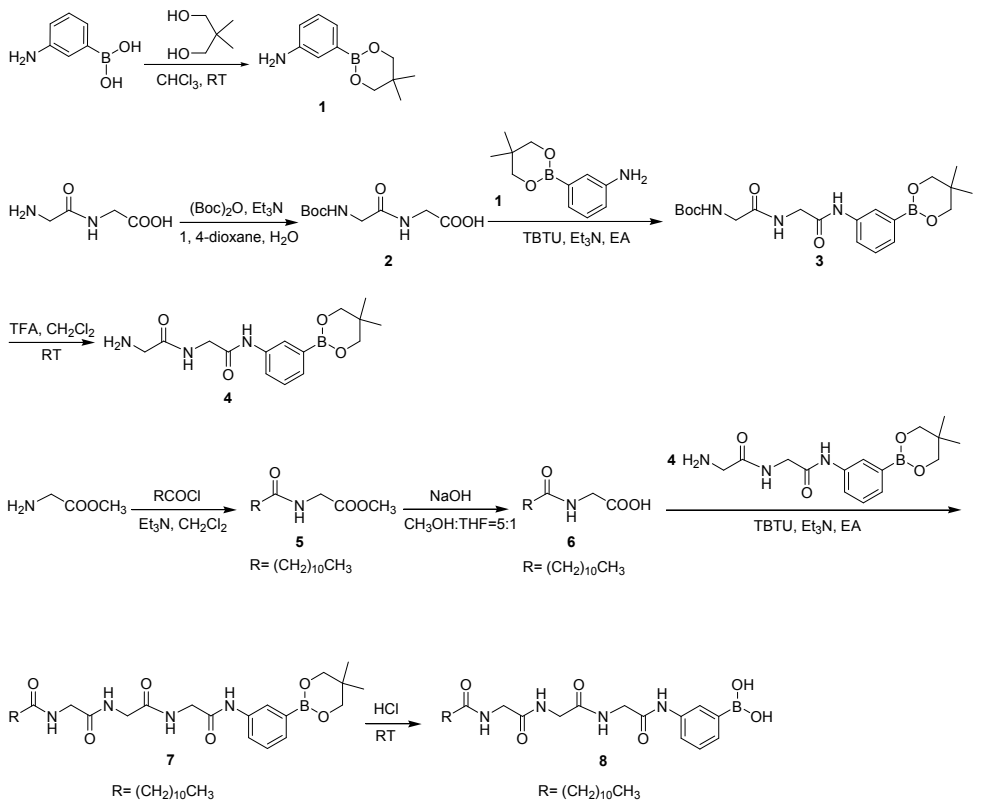
Immunofluorescence staining

After the MSCs being cultured for 14 days, the cells were fixed for 20 min in a 3.7% formaldehyde solution. Cell membranes were permeated by soaking with 0.5% Triton-X100 for 10 min at 4 °C. The samples were blocked the bovine serum albumin for non-specific binding and then incubated

with primary antibody against collagen II (1:200 dilution in 0.1%BSA/PBS; Rabbit anti-collagen II; Abcam, UK) or OCN (1:200 dilution in 0.1%BSA/PBS; Mouse anti-collagen II; Abcam, UK) overnight at 4 °C. Secondary antibody labeling was performed with collagen II antibody (1:250 dilution in 0.1%BSA/PBS; FITC conjugated goat anti-rabbit Ig G; Bioss, China) and OCN antibody (1:250 dilution in 0.1%BSA/PBS; FITC conjugated goat anti-mouse Ig G; Bioss, China), incubating at 37 ˚C for 1 h, respectively. Before observation, the MSCs were stained with 10 μg/ml of Hochest 3342 for 5 minutes.

Statistical analysis

All data were expressed as means values ± standard deviation for all experiments. Differences between the various groups were analyzed by the one-way ANOVA test with Bonferoni's method using the programme SPSS. The level of significance was set at $p< 0.05$.



Scheme S1. The synthesis of low molecular weight gelator (compound 8).

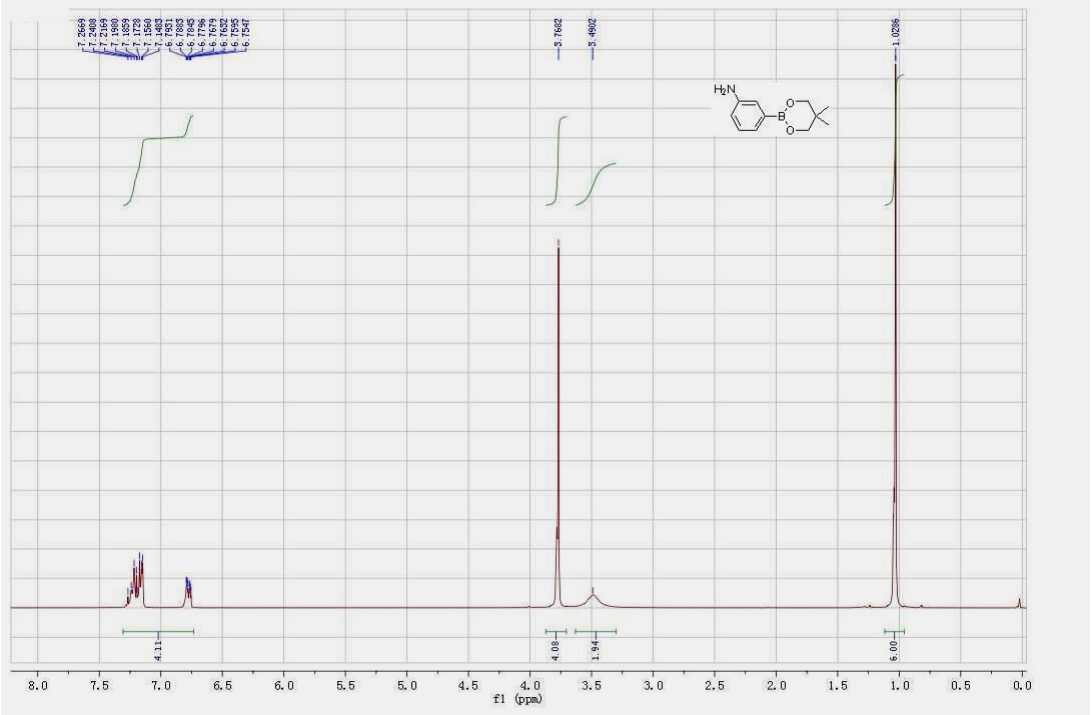


Figure S1. The ^{1}H NMR spectrum of compound 1.

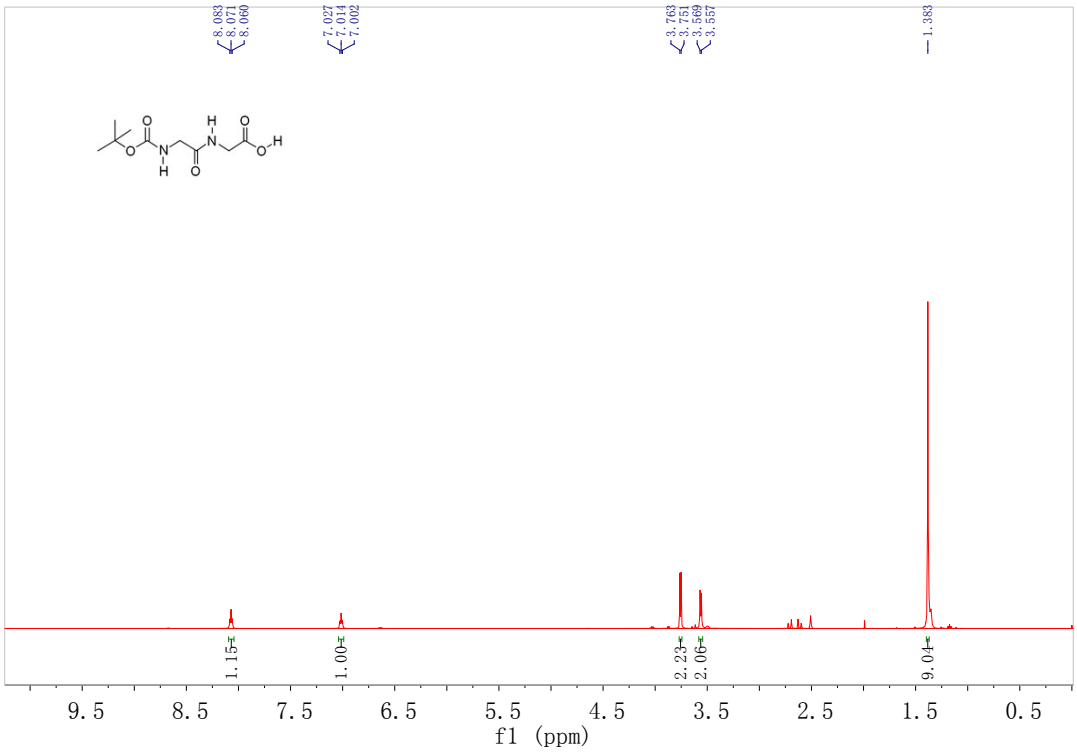


Figure S2. The ^{1}H NMR spectrum of compound 2.

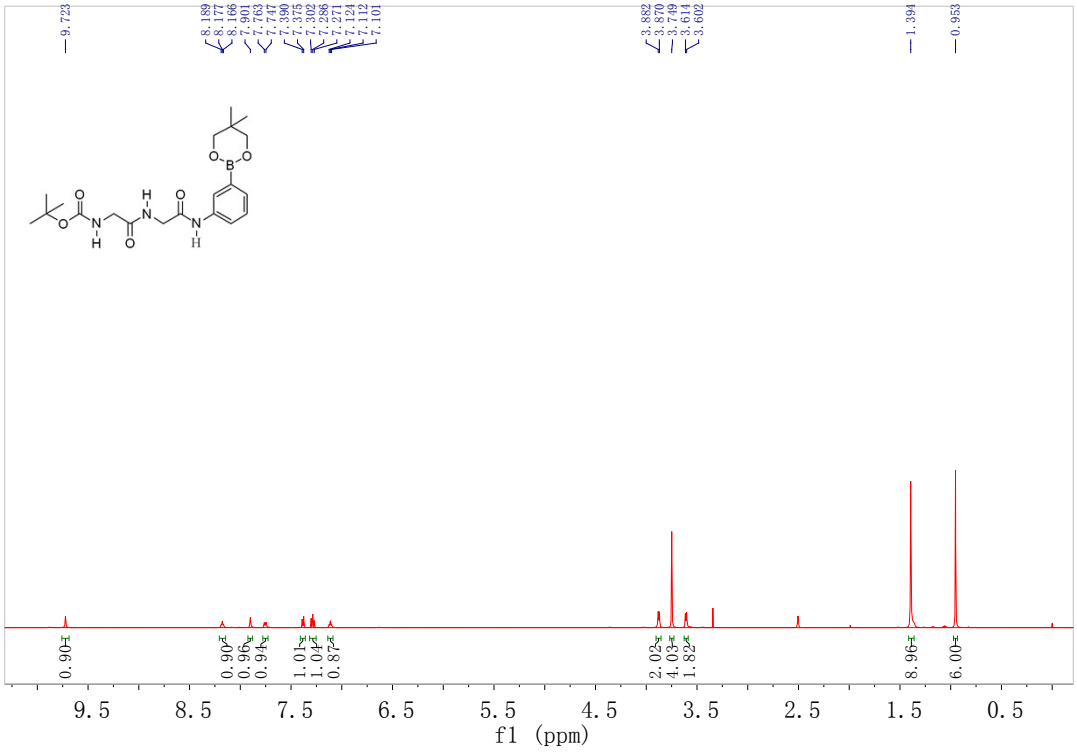


Figure S3. The ^{1}H NMR spectrum of compound 3.

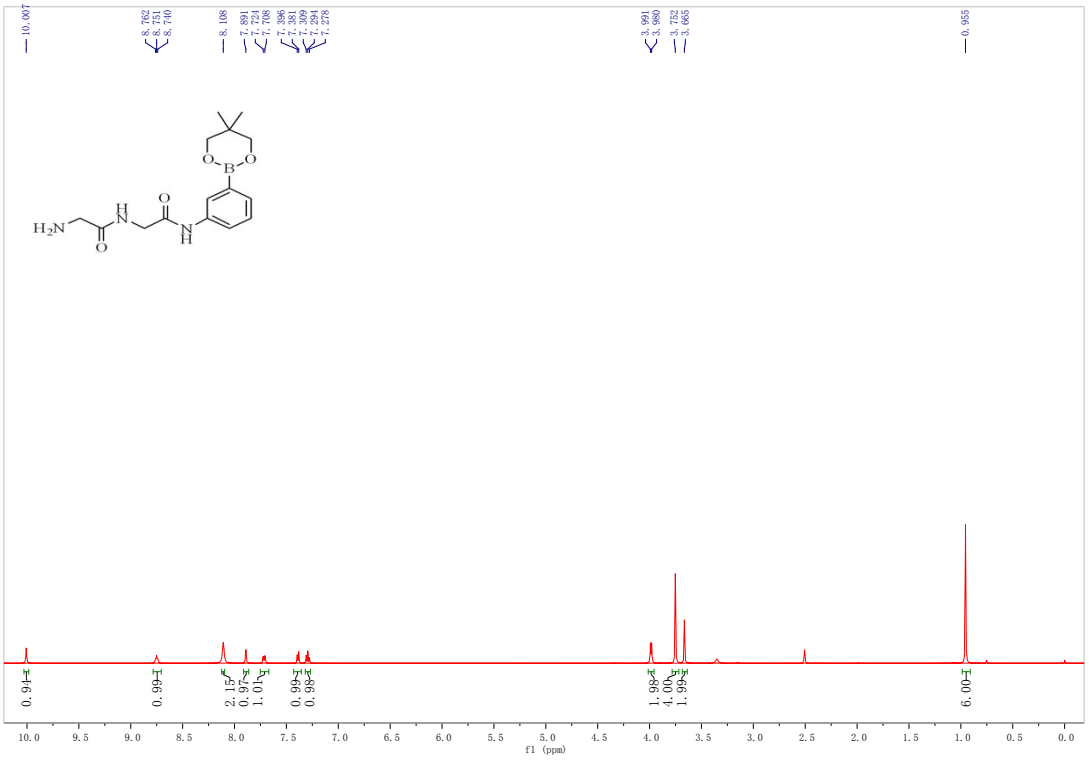


Figure S4. The ^{1}H NMR spectrum of compound 4.

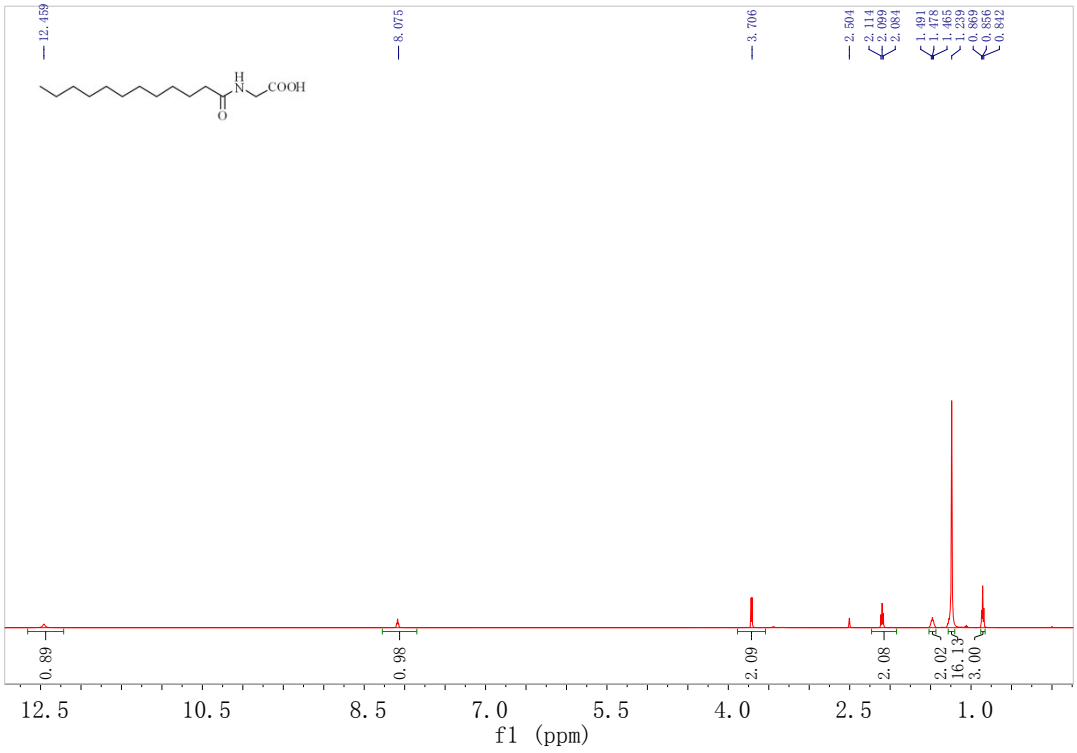


Figure S5. The ^{1}H NMR spectrum of compound 6.

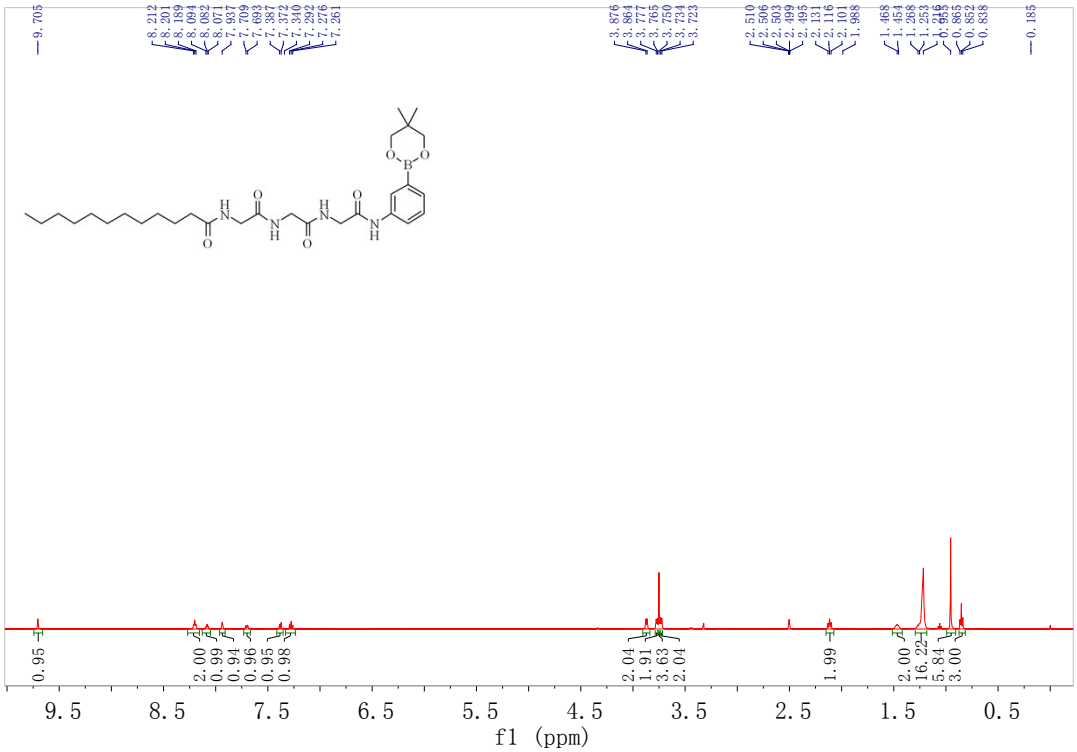


Figure S6. The ^{1}H NMR spectrum of compound 7.

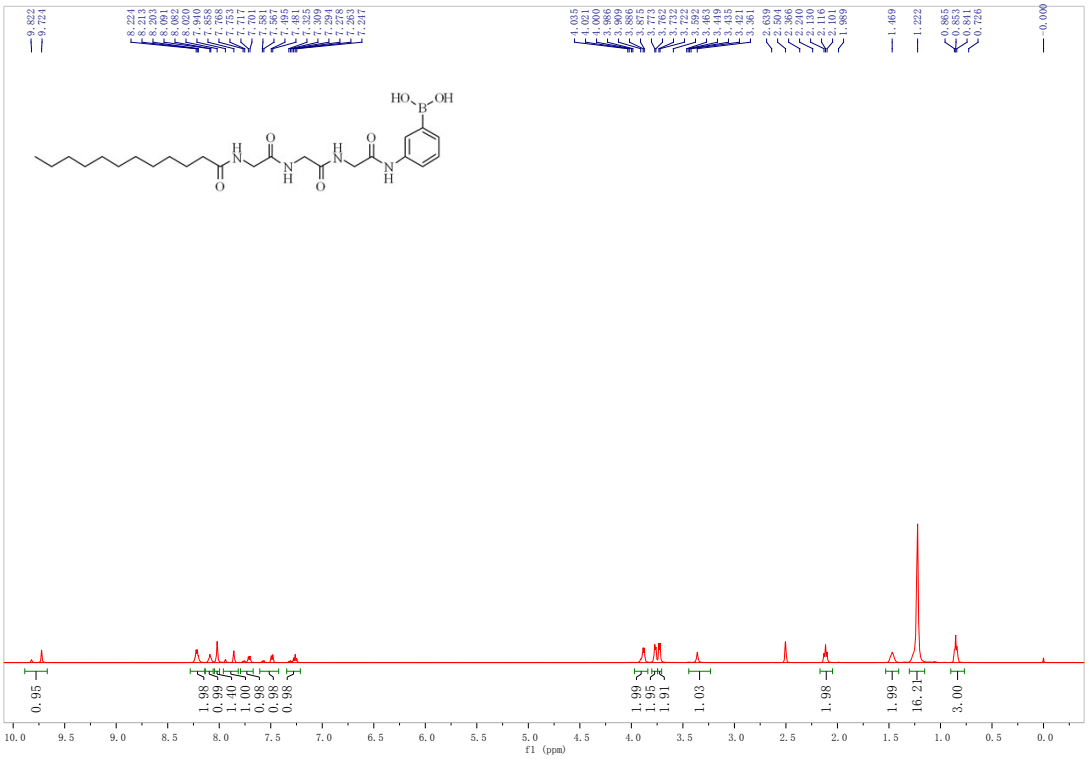


Figure S7. The ^{1}H NMR spectrum of compound 8.

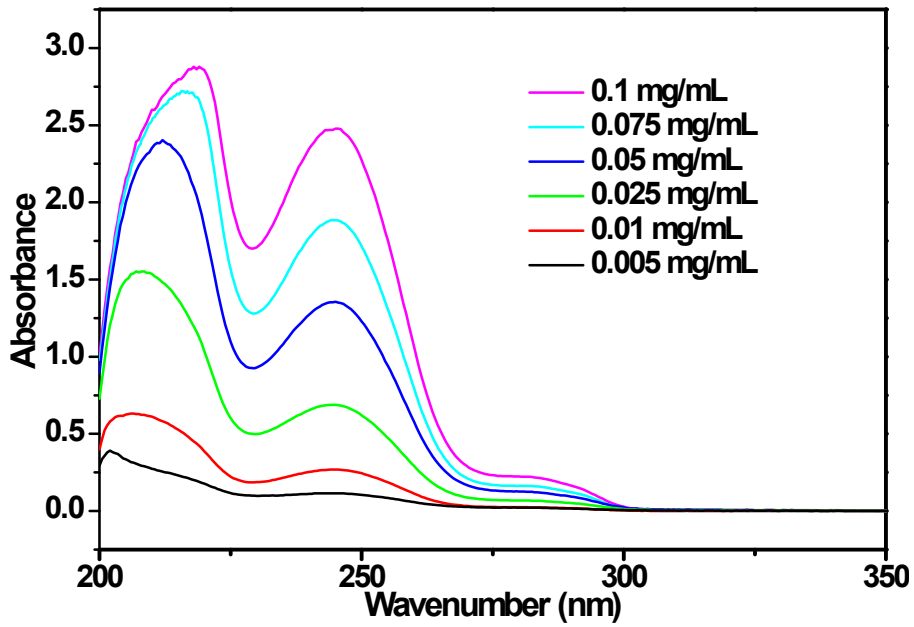


Figure S8. The formation of π-π interaction within the gel with increasing the concentration.

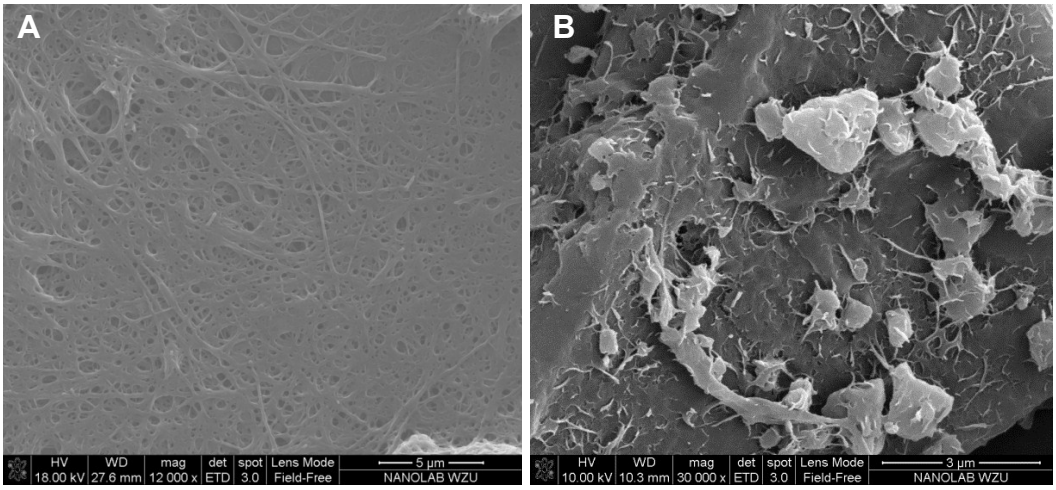


Figure S9. The morphologies of xerogels, A: gel prepared in isopropanol; B: gel prepared in PEG200:H_2O=4:1.

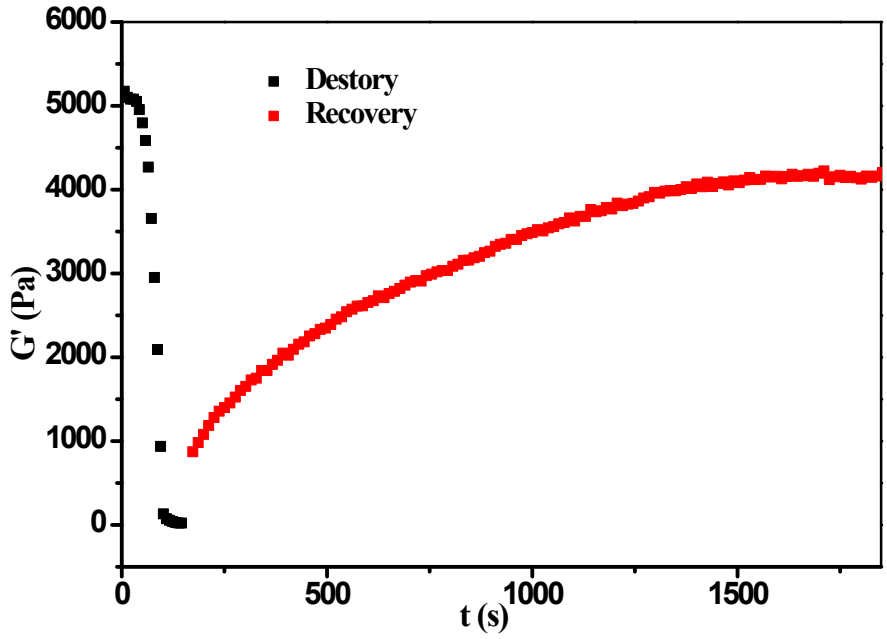


Figure S10. Recovery of gel of compound 8 in PEG200:H_2O=4:1 (at 25 °C): 16 mg/mL, the gel was first subjected to a large strain of 50% for 100s and then its recovery was probed at the strain of 0.1% and frequency of 1 rad/s.

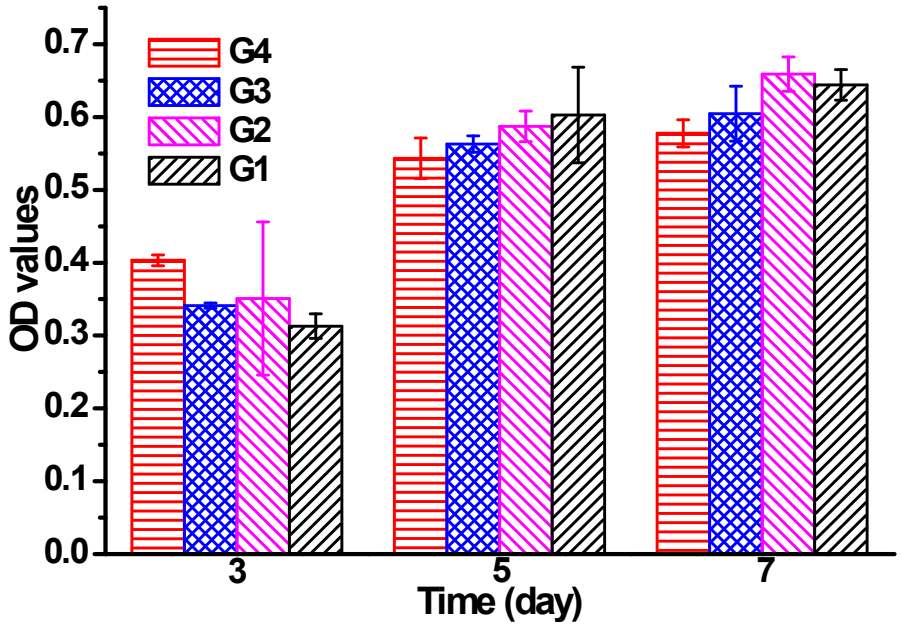


Figure S11. The proliferation of MSCs in the gels incubated with different time. No significant difference was found between the gels with the same incubation time via statistic calculation.

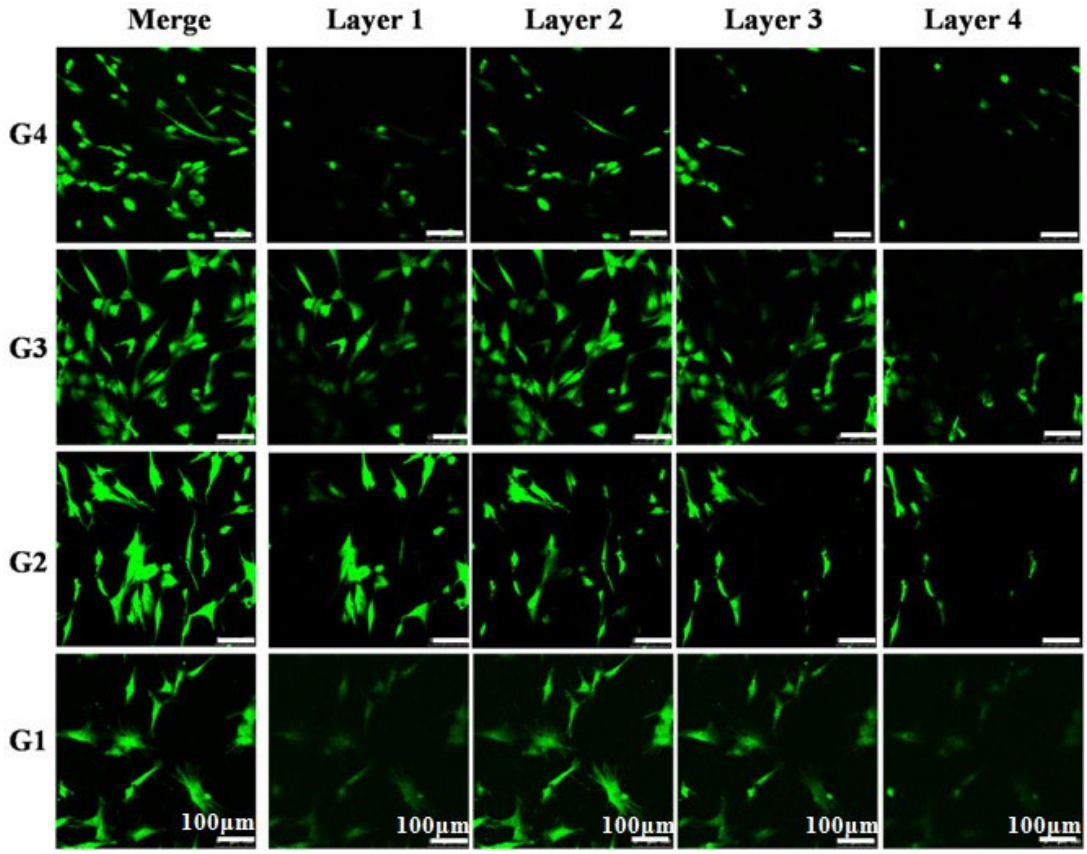


Figure S12. The morphology of MSCs in the gels under confocal laser scanning microscopy.