Supplementary materials

Materials and Reagents

Gelatin from porcine skin, type A (300 bloom, MW: 50k~100k), Hexanoic anhydride (HA, 97%), Benzyl ether (99%), Fetal bovine serum, Trypsin-EDTA, Bovine serum albumin (BSA), Alcian Blue 8GX powder, and Insulin-transferrin-sodium liquid media supplement (ITS, 100X) were purchased from Sigma Chemical Co. Iron (III) acetylacetonate, 1, 2-Hexadecanediod (97%), Oleic acid (90%), Oleylamino (>70%), Chloroform, Rhodamine, Span 80, and Tween 80 were obtained from Aldrich Co. CdSe/ZnS core/shell quantum dot solid (Q.D. 620nm, 50mg) was purchased from OceanNanoTech. Human retinal pigmented epithelium cells (ARPE-19) were obtained from the Bioresource Collection and Research Center in Taiwan. All other reagents including Formadehyde (Riedel-de Haën), Triton-X 100 (J.T. Beker), Dimethyl sulfoxide (DMSO, J.T.Baker), 4'-6-Diamidino-2-phenylindole (DAPI, Invitrogen), Rhodamine Phalloidin (Fluka) Fluorescence Mounting Medium (Dako), Alex Fluor ® 488 Phalloidin (Thermo Fisher) Collagen type I antibody (GeneTex), Collagen type II antibody (GeneTex and R&D system), Aggrecan antibody (GeneTex), and Rabbit IgG antibody (FITC, GeneTex) were of analytical grade. Recombinant mouse transforming growth factor protein beta 1, and quantikine ELISA kit (Mouse/Rat/Porcine/Canine TGF-β1) were purchased from R&D system. RNAzol®RT was from Molecular Research Center, Inc., and GScript First-Strand Synthesis Kit as well as 50bp DNA Ladder RTU were from GeneDireX Inc. The Platium® Blue PCR SuperMix was from Invitrogen, agarose was purchased from Focus Bioscience, Sybr® Safe DNA gel stain was from Thermo Fisher, and Kapa Sybr Fast qPCR kit was purchased from Roche® LightCycler 480, Kapa Biosystem.

Illustration and NMR analysis of gelation and amphiphilic gelatin

The chemical structures of the primary and modified gelatin are illustrated in **Figure. S1**. The ^{1}H NMR spectra of primitive gelatin molecules and the modified gelatin are shown in Figure S2(a) and (b), respectively**.** There were three different shift signals between primary and modified gelatin marked with a star sign and letters A and B. In comparison to the signals with primary gelatin spectra, peak A and B are the additional chemical shift signals on modified spectra matching the signal of the hexanoyl group ($COC_{\alpha}H_2$, around 2-2.3) and methyl group (CH_3, below 0.9) provided by hexanoic anhydride. Additionally, the substitution from amino group to hexanoyl group is confirmed by the star-signed peaks. The signal intensity at 2.8 ppm assigned to the primary amino group was much reduced from its original site, and the chemical shift became broader by integral calculation, which indicates that the chemical linkage between the gelatin backbone and hydrophobic carbon chain was successful.

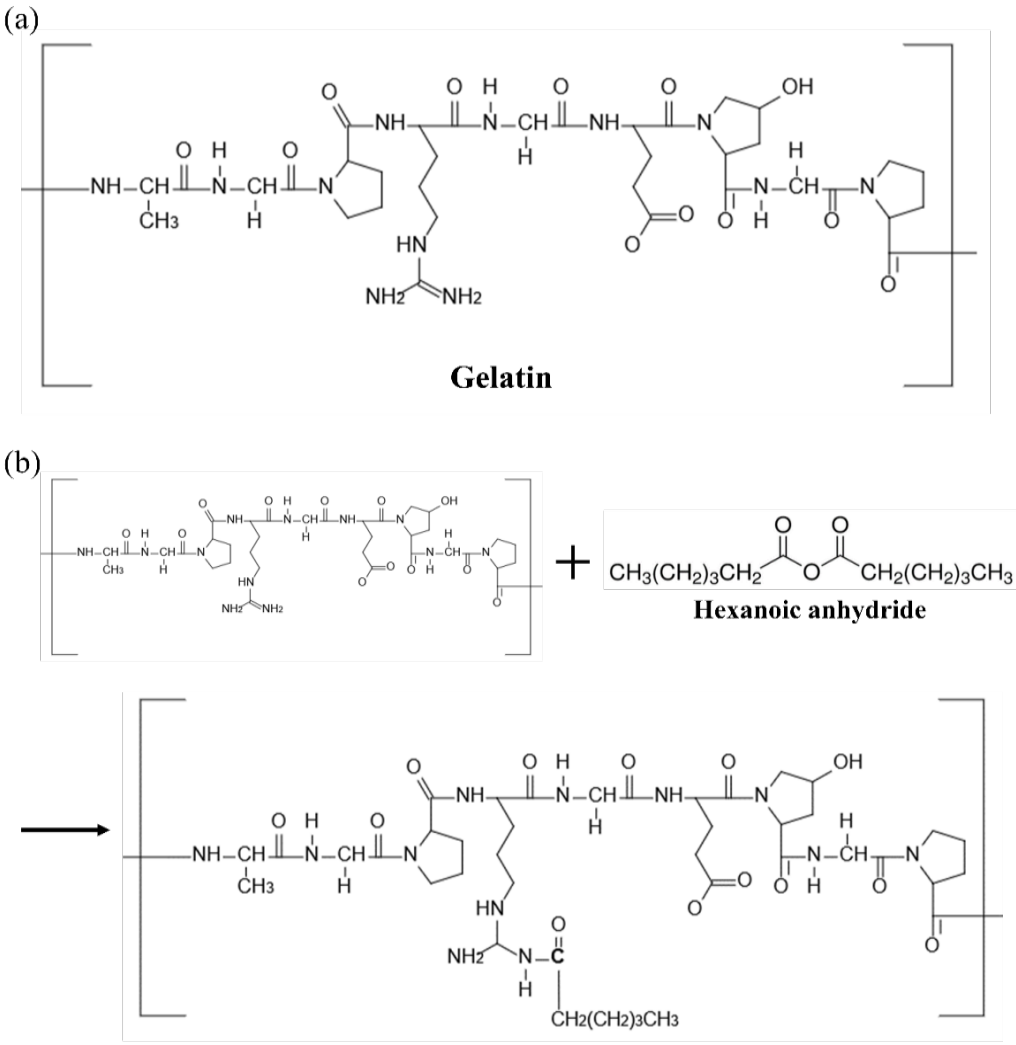

Fig. S1 Illustration of gelatin and amphiphilic gelatin: (a) gelatin monomer and (b) the reaction scheme for the synthesis of amphiphilic gelatin.

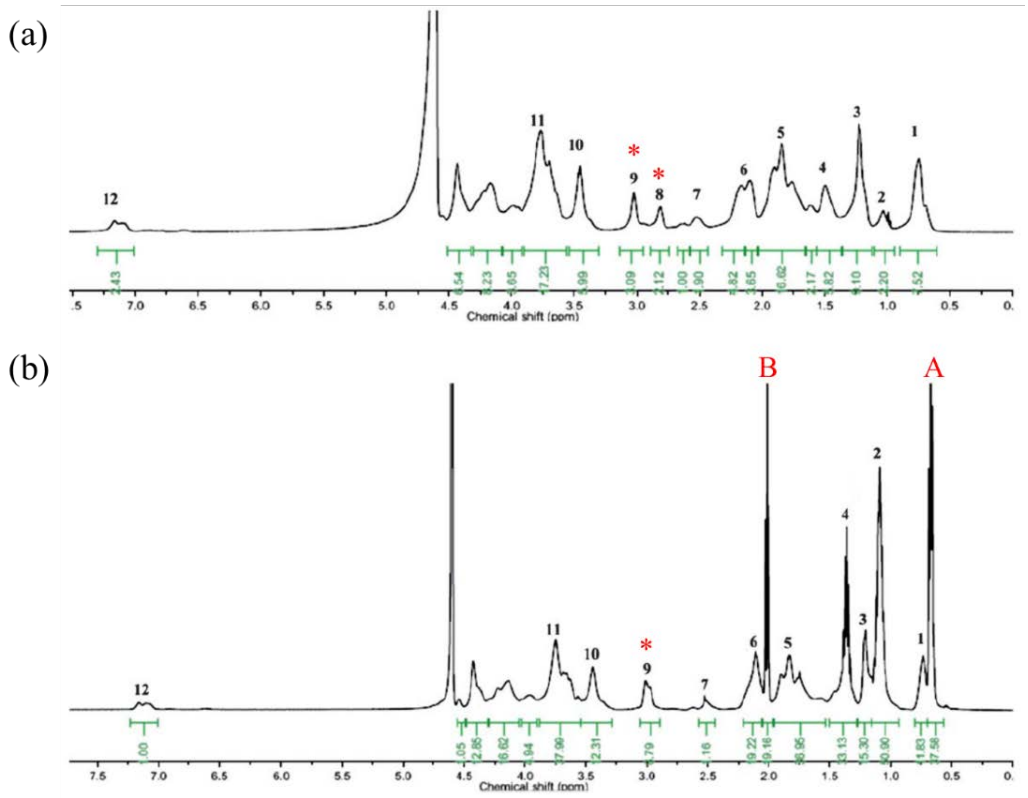

Fig. S2 ^{1}H-NMR spectrum of gelatin molecules in D_2O: (a) primitive gelatin molecules, and (b) modified gelatin molecules.

Primer sequence

Table S1 Primer sequence of cartilage expression genes in ATDC5 cells

Type I collagen	GAGCCTGAGTCAGCAGATTGAGAAC	forward
	GCTGAGTAGGGAACAC ACAGGTCTG	reverse
Type II collagen	CGTGGACGCTCAGGAGAAACAG	forward
	CAGTGACTTGAGTGTAGCGTCCACC	reverse
Aggrecan	CAAAAATGCTCAAGACTACCAGTGGATC	forward
	CCAGATCATCACC ACACAGTCCTCTC	reverse
GAPDH	GATGACATCAAGAAGGTGGTGAAGC	forward
	CCGTATTCATTGTCATACCAGGAAATG	reverse

Blyscan assay

A Blyscan assay was performed to examine the sGAG secretion of ATDC5 cells. The standard curve of the Blyscan assay was completed in a dilution series of glycosaminoglycan standard solution (100 μg ml^{-1}). To measure the amount of sGAG, the culture media (1 ml) from ATDC5 cells treated with different MAGNCs formulations was collected and centrifuged at 12000rpm for 10 min in a sterile microfuge tube. After centrifugation, the supernatant (500 μl) was mixed with standard solution, and then Blyscan dye reagent (500 μl) was added and the mixture was vortexed for 30 sec and incubated for 30 min. The insoluble sGAG-dye complex formed at the bottom of the tubes was dissolved by adding dissociation reagent (500 μl), and the solution (200 μl) was then transferred to 96-well plates and absorbance was measured at 656 nm.

Gene expression

ATDC5 cells were cultured in 10 cm culture dish for all experiment groups. To extract the RNA, the media was removed and the RNAzol® RT reagent (1ml) was added to facilitate cell lysis. The lysate was collected in a sterile microfuge tube, mixed with DEPC-treated water (0.4 ml), and vortexed vigorously for 15 sec. After incubated at 4°C for 15 min, the sample was centrifuged for 15 min at 12000 rpm. The supernatant (1 ml) was transferred to a new sterile microfuge tube, and the mRNA was precipitated by adding 75% ethanol (0.4 ml). After washing with 75% ethanol, the mRNA pellet was collected under centrifugation, and reconstituted using DEPC-treated water (20 μl) for further measurement.

Before measurement, the concentration of total mRNA was determined by nanophotometer. The normalized samples with the same mRNA amount were mixed with $Oligo(dT)_{20}$ (50 μM, 1 μl), dNTP mix (10 mM, 1 μl), and DEPC-treated water. The solutions were vortexed, heated for 5 minutes at 65ºC in thermal cycler, and placed promptly on ice. Afterward, the 5X 1st strand buffer (4 μl), DTT (0.1M, 1μl), GScript RTase (1 μLl), and

DEPC-treated water were added to the tubes, and the mixed solutions were treated with a sequential thermal process for 60 min at 50°C, 15 min at 70°C, and then 4°C. The final samples were stored at -20°C for PCR and qPCR usage.

Briefly, the cDNA sample from reverse transcription (100 ng) was mixed with Platinum® Blue PCR SuperMix (45 μl) and forward/reverse primer (10 μM, 0.5 μl). The solution was heated to 95°C for 5 min, and then the PCR reaction was cycled in set-up temperatures for 30 sec at 95°C, 30 sec at 55°C, and 30 sec at 72°C, for 35 cycles. After the PCR cycle ended at 72°C for 3 min and cooled down to 4°C, the samples were stored at 4°C and PCR products were separated by electrophoresis. The primer sequences of type I collagen (*Col1a1*), type II collagen (*Co2a1*), aggrecan (*ACAN*), and GAPDH are shown in **Table S1.**

Relationship between structural stability and SPIO concentrations

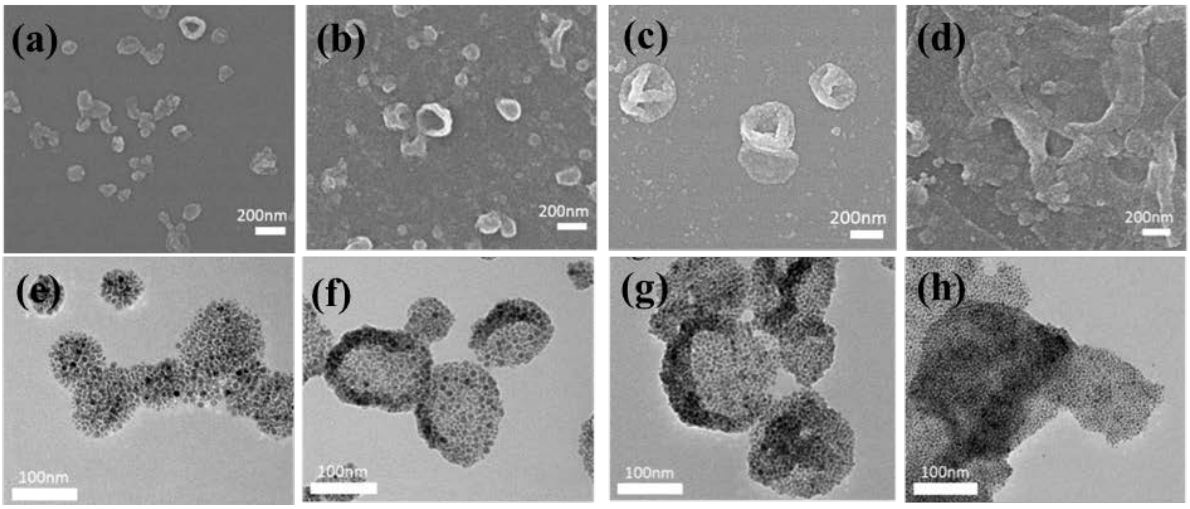

Fig. S3 SEM (a, b, c, d) and TEM (e, f, g, h) pictures of different initial concentrations of SPIO, which result in different sizes and conformations of emulsion with amphiphilic gelatin. SPIO concentrations: (a,e) 3 mg, (b,f) 5 mg, (c, g) 10 mg, and (d, h) 30mg.

Size distribution of the MAGNCs

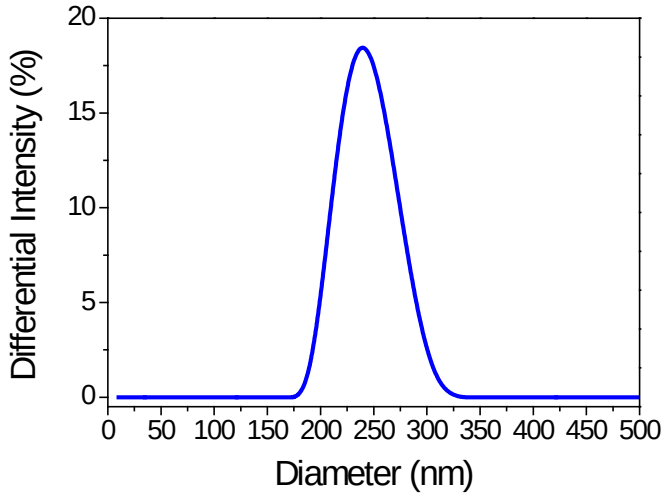

Fig. S4 The sizes of the MAGNCs were measured to be 200-300 nm using a dynamic light scattering instrument (Beckman Coulter Delsa™ Nano C particle analyzer).

Cell viability of ATDC5 after long-term MAGNC incubation

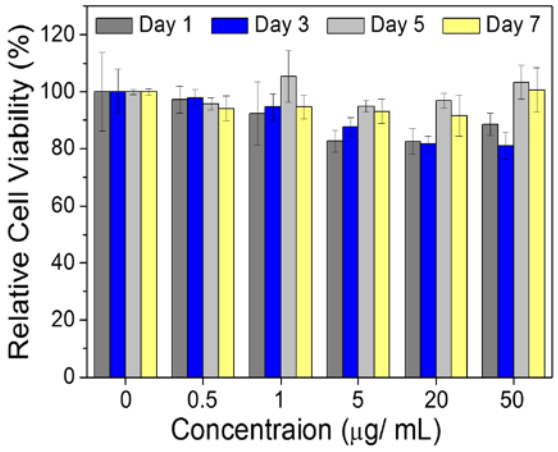

Fig. S5 The relative cell viability of ATDC5 incubated with different concentrations of MAGNCs for 1-7 days.

Differentiation of ATDC5 cells

The samples on the left side of the gel in **Figure S6(a)** represent the negative control (ctrl-), and the samples on the right side represent the positive control (ctrl+). The ctrl+ group had a higher concentration of *Col2a1* expression than the ctrl- group after 7 days and 14 days of treatment; *Col1a1* expression remained the same for all groups, indicating that rmTGF-β1 has a positive effect on type II collagen secretion on ATDC5 cells, and rmTGF-β1 does not induce dedifferentiation resulting in increased type I collagen secretion. In **Figure S6(b)**, Alcian blue staining shows the sGAG secretion of the ctrl+ group at 7 and 14 days, and the sGAG expression is higher in 14 days for a wider spreading of blue stained area. In the end, we examined the exclusive protein secretion of ATDC5 cells by immunofluorescence staining to determine the expression level of type II collagen under stimulation. In **Figure S6(c)**, the type II collagen expression level at the ctrl+ group gradually increased and showed an obviously higher level compared to that in the ctrl- group at 14 days.

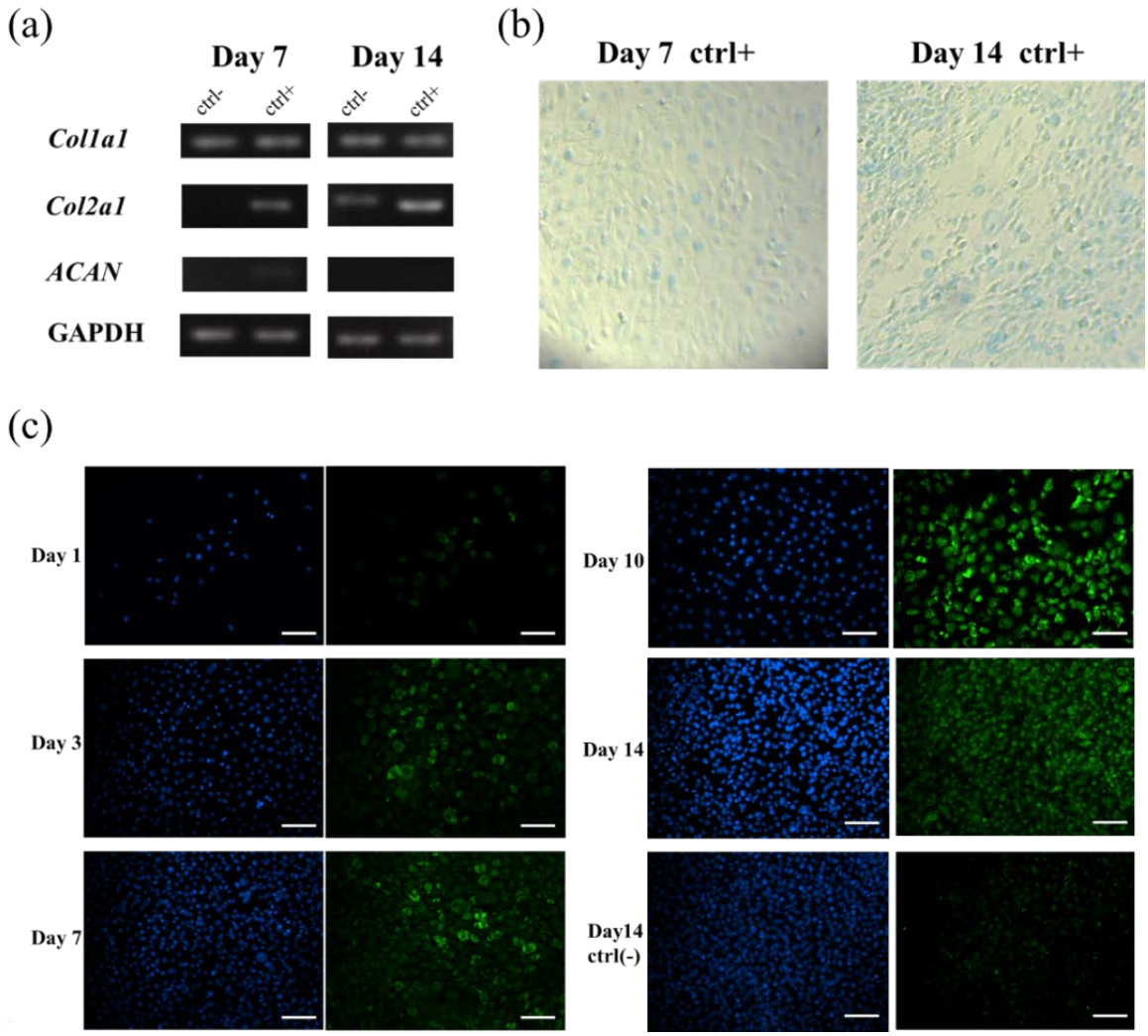

Fig. S6 Evaluation of ATDC5 differentiation by (a) PCR, (b) Alcian blue stain to assess sGAG, and (c) immunofluorescence stain for collagen type II detection (scale bar: 100 μm).

Observation of type II collagen expression

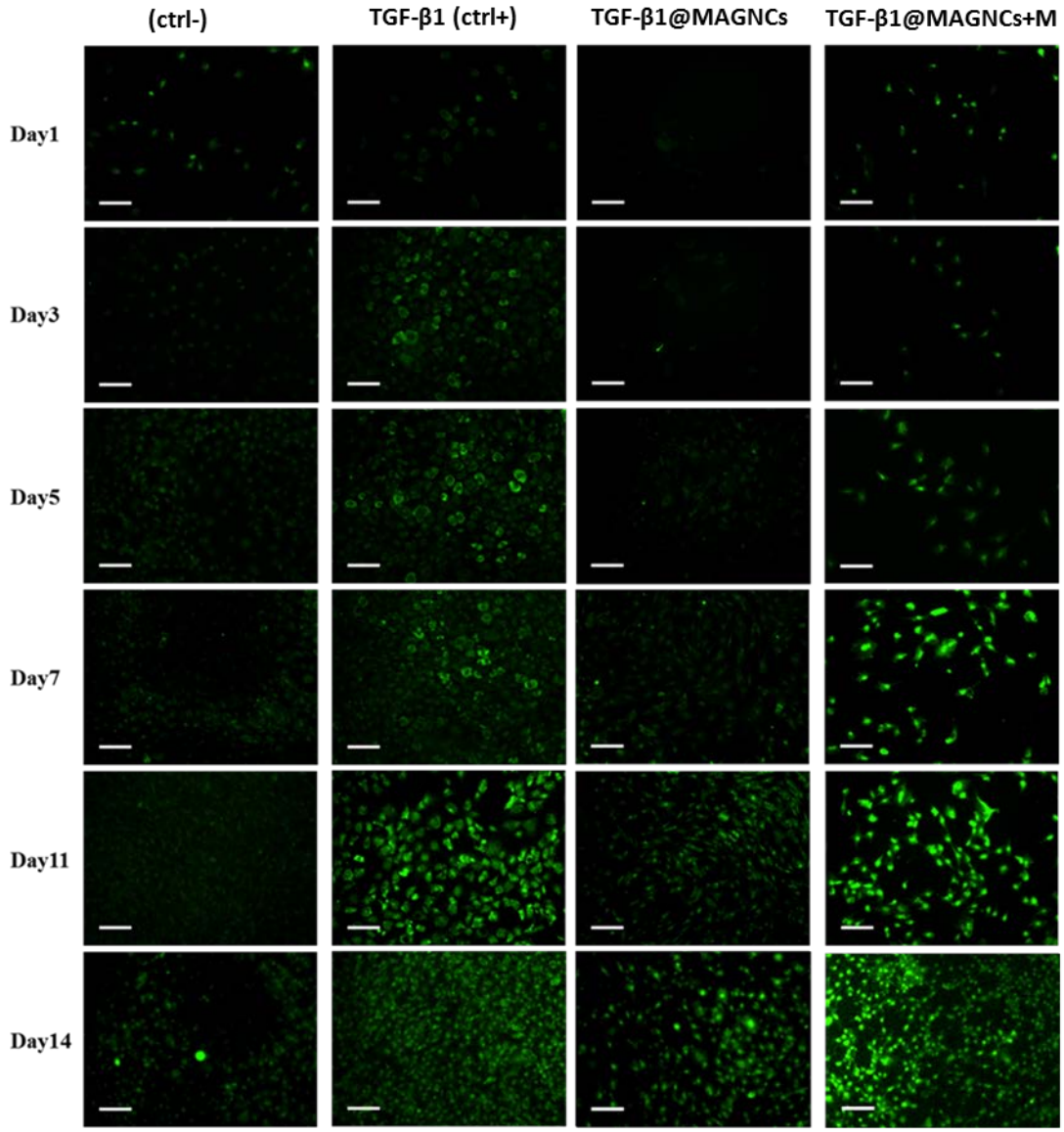

Fig. S7 Immunofluorescence staining of mouse type II collagen with FITC-labeled antibody presenting the relative expression level under different stimuli for 1-14 days of observation. (Scale bar: 100 μm)