Supporting Information

for

Sulfonated glycosaminoglycan bioinspired carbon dots for cellular labelling and effective promotion on the differentiation of mesenchymal stem cells

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Figures

Fig. S1 Fluorescence intensity of synthesized CDs under different (a) carbonization temperature, (b) carbonization time and (c) molar ratios of GA•HCl/NaSS.

Fig. S2 (a) Dynamic light scattering (DLS), (b) fourier transform infrared (FT-IR) and (c) zeta potential characterization of synthesized CDs.

Fig. S3 High resolution X-ray photoelectron spectroscopy (XPS, including (a) C_{1s}, (b) N_{1s}, (c) O_{1s} and (d) S_{2p} spectra) of synthesized CDs.

Fig. S4 Stability characterization of synthesized CDs in the aqueous solution under different conditions.

Fig. S5 Confocal images of rBMSCs cultured with CDs (*conc.*=25 μg/mL) for different cultivation intervals (1, 6 and 24 h).

Fig. S6 Confocal images of rBMSCs obtained in a randomly chosen area (10 cells in area) after 24 h cultivation in the presence of CDs (*conc.*=25 μg/mL).

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Fig. S9 (a) ALP activity (nmol/min) and (b) total protein expression (mg) of rBMSCs in different cultures during the osteogenic differentiation.

Fig. S10 Bright field images of rBMSCs cultured in different medium (BM and OIM without and with CDs) for 3, 7 and 14 days.

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Fig. S12 (a) ALP activity and (b) Col I expression (relative to BM) of rBMSCs cultured in different mediums (BM without and with CDs) during the differentiation times; (c)

optical microscope images of calcium nodules stained with alizarin red S after the cultivation in different mediums for 7 and 14 days.

Fig. S13 Bright field images of rBMSCs cultured in BM without and with CDs for 3, 7 and 14 days.

Fig. S14 Bright field images of rBMSCs cultured in BM and CIM without and with CDs for 3, 7 and 14 days.

Fig. S15 Optical images of toluidine blue O staining after rBMSCs cultured with different mediums for 7 and 14 days.

Fig. S16 (a) GAG and (b) Col II expression (relative to BM) of rBMSCs cultured in different mediums (BM without and with CDs) during the differentiation times; (c) optical microscope images of GAG stained with toluidine blue O after the cultivation in different mediums for 7 and 14 days.

Fig. S17 Intracellular ROS detection of rMSCs cultured in different culture media (BM, OIM and OIM+CDs) for different days (7, and 14 d). DCFH-DA as ROS sensor, scale bar=200 μm.

Fig. S18 Intracellular ROS detection of rMSCs cultured in different culture media (BM, CIM and CIM+CDs) for different days (7, and 14 d). DCFH-DA as ROS sensor, scale bar=200 μm.

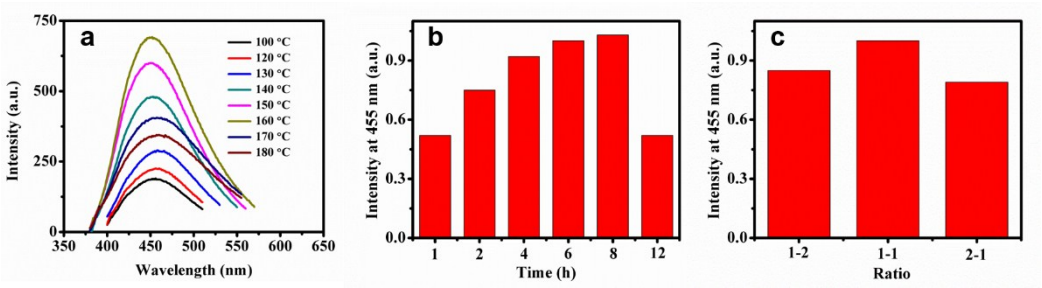

Fig. S1 Fluorescence intensity of obtained CDs under different (a) carbonization temperature, (b) carbonization time and (c) initial feed ratios of GA•HCl/NaSS (intensity at 6 h or ratio of 1-1 was normalized as 1).

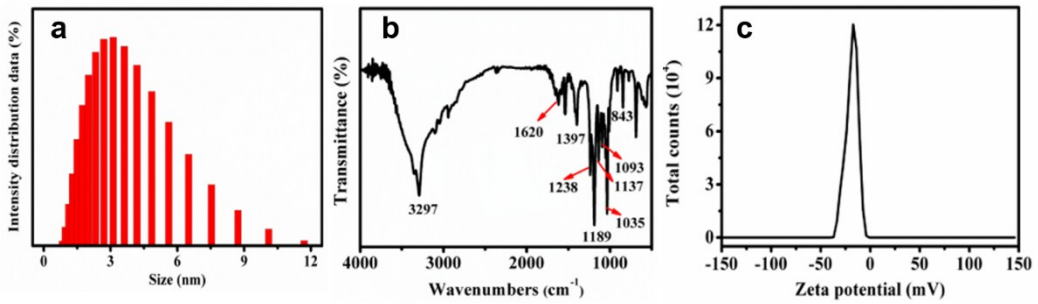

Fig. S2 (a) Dynamic light scattering (DLS), (b) fourier transform infrared (FT-IR) and (c) zeta potential characterization of synthesized CDs.

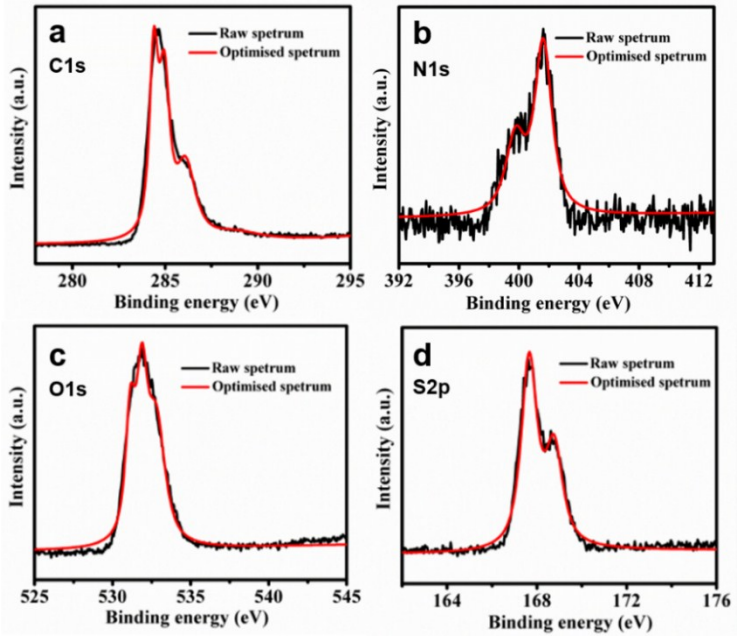

Fig. S3 High-resolution (a) C_{1s}, (b) N_{1s}, (c) O_{1s} and (d) S_{2p} X-ray photoelectron spectroscopy (XPS) of synthesized CDs.

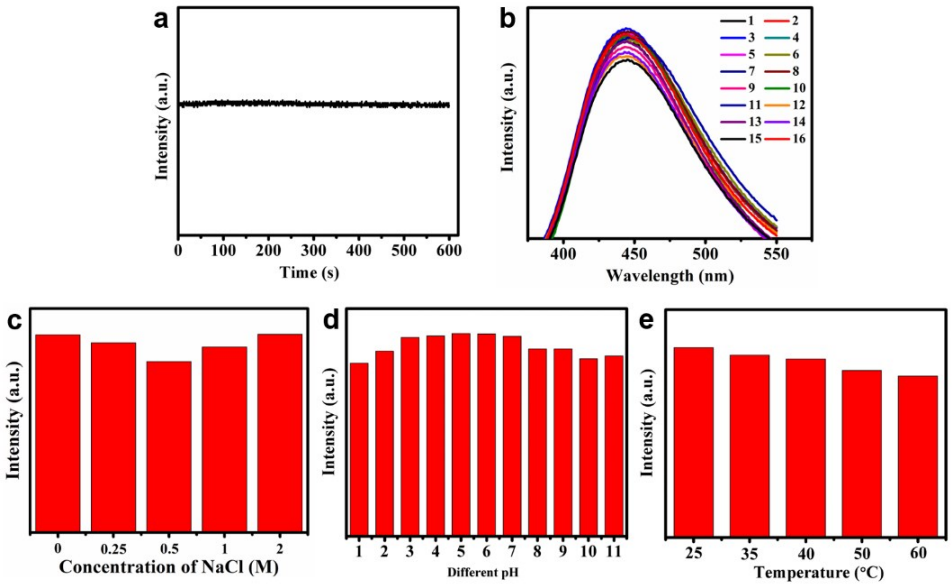

Fig. S4 Stability characterization of CDs solution (*conc.*=0.25 mg/mL) in the conditions of (a) continuous UV irradiation (λ=378 nm) lasting for 10 min, (b) mixing with cations, anions and anime acids solution (from 1 to 16, CDs solution (0.25 mg/mL, blank), Fe^{3+}, Fe^{2+}, Ag^{+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, CO_3^{2-}, HCO_3^{-}, HPO_4^{2-}, $H_2PO_4^{-}$, PO_4^{3-}, Asp, Phe, Tyr, Arg: *conc.*=1 mM), (c) NaCl solution with different concentrations, (d) dispersion in the solutions of different pH values and (e) heat treatment at different temperatures ranging from 10 to 60 °C.

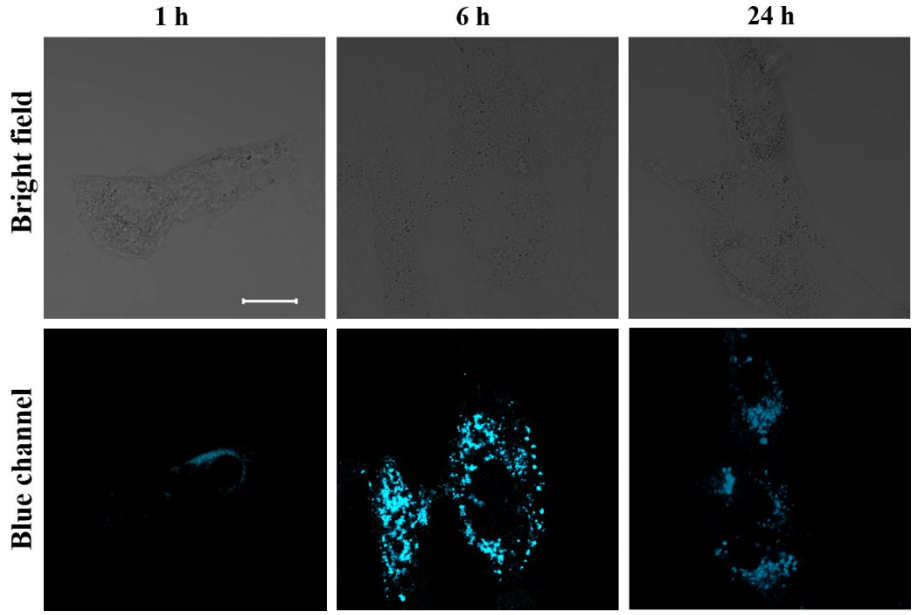

Fig. S5 Confocal images of rBMSCs cultured with CDs (*conc.*=25 μg/mL) for different cultivation intervals (1, 6 and 24 h), Scale bar = 20 μm.

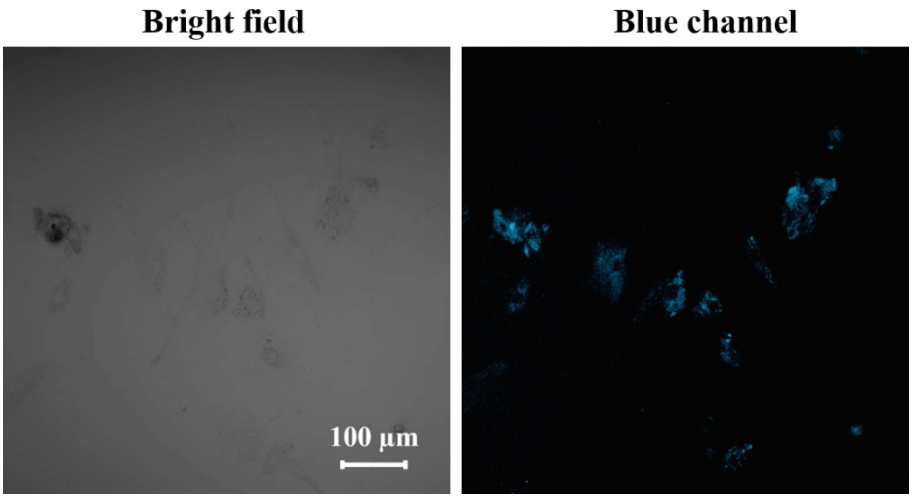

Fig. S6 Confocal images of rBMSCs obtained in a randomly chosen area (10 cells in area) after 24 h cultivation in the presence of CDs (*conc.*=25 µg/mL).

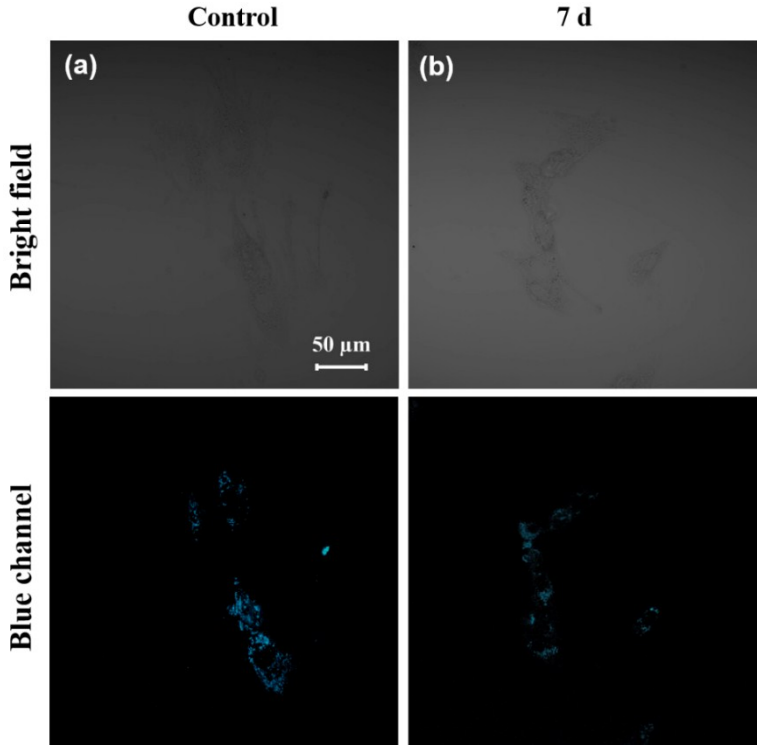

Fig. S7 Confocal images of rBMSCs (a) cultured in BM with CDs (*conc.*=25 µg/mL) for 24 h (Control) and (b) subsequently cultured in BM without CDs for 7 days.

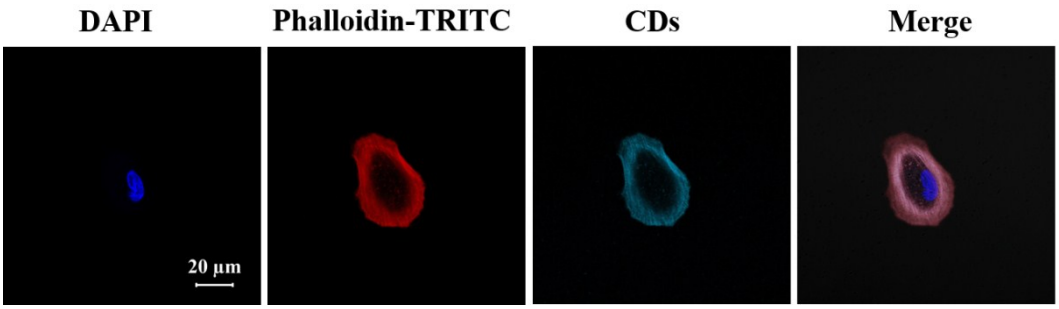

Fig. S8 Confocal images of rBMSCs cultured with CDs (*conc.*=25 μg/mL) for 12 h (cell nucleus and actin cytoskeleton were stained with DAPI, phalloidin-TRITC and synthesized CDs respectively).

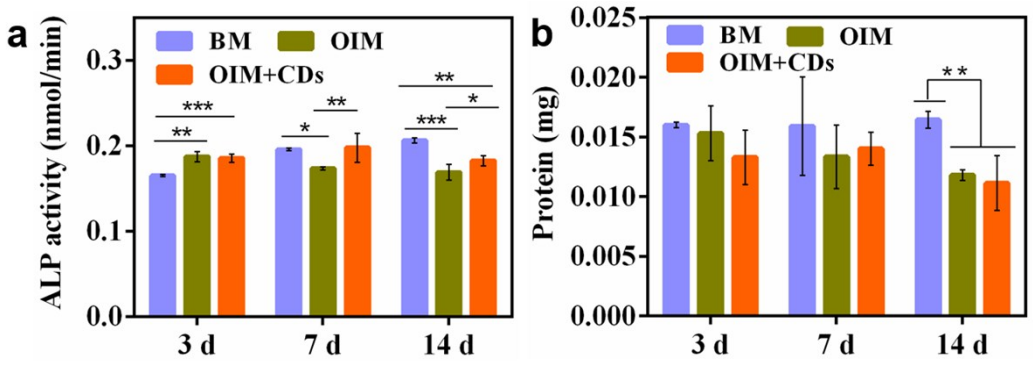

Fig. S9 (a) ALP activity (nmol/min) and (b) total protein expression (mg) of rBMSCs incubated in different mediums without and with CDs (*conc.*=25 μg/mL) for 3, 7 and 14 days (mean values ± SD, ***p < 0.001, **p < 0.01, *p < 0.05).

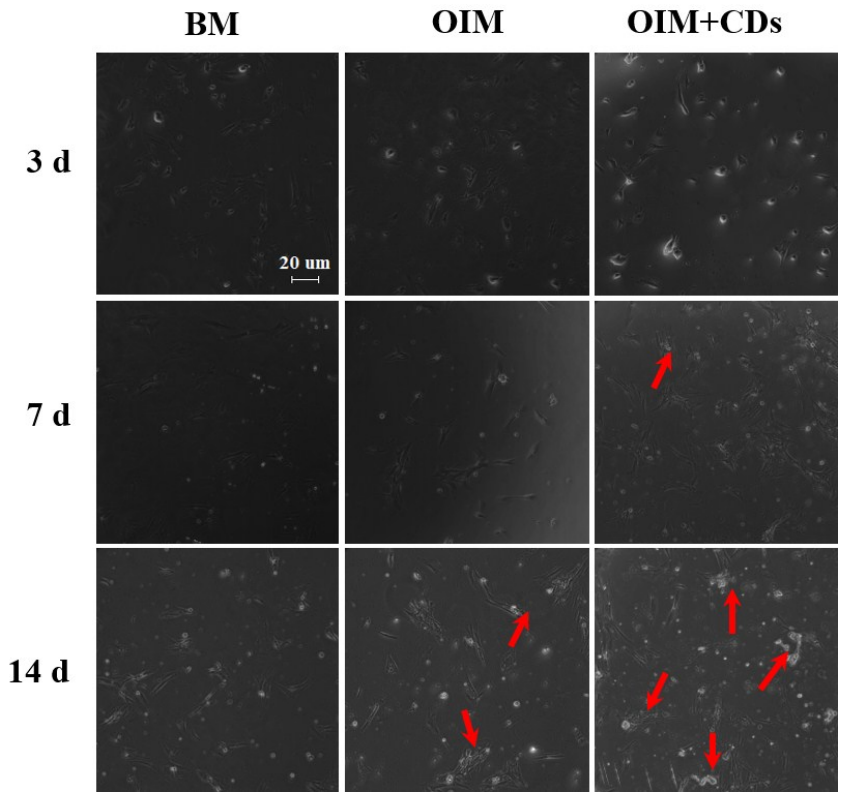

Fig. S10 Bright field images of rBMSCs cultured in different mediums without and with CDs (*conc.*=25 μg/mL) for 3, 7, and 14 days (red arrows showed calcium nodules).

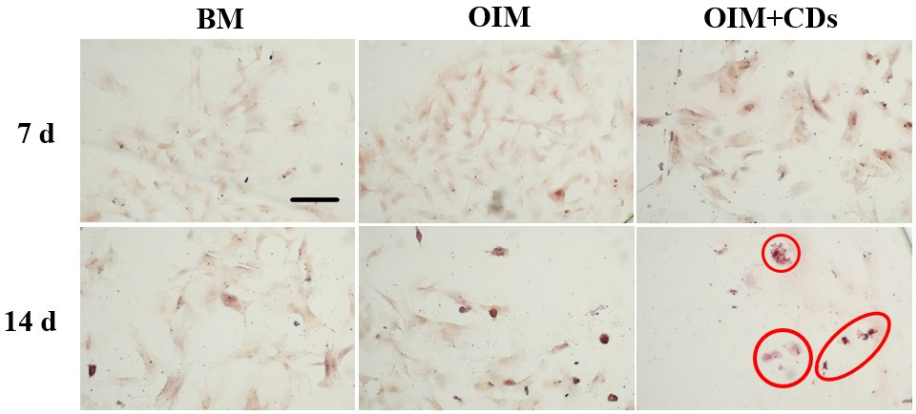

Fig. S11 Optical microscope images of calcium nodules stained with alizarin red S after rBMSCs cultured in different mediums for 7 and 14 days, CDs: *conc.*=25 μg/mL, scale bar = 200 μm.

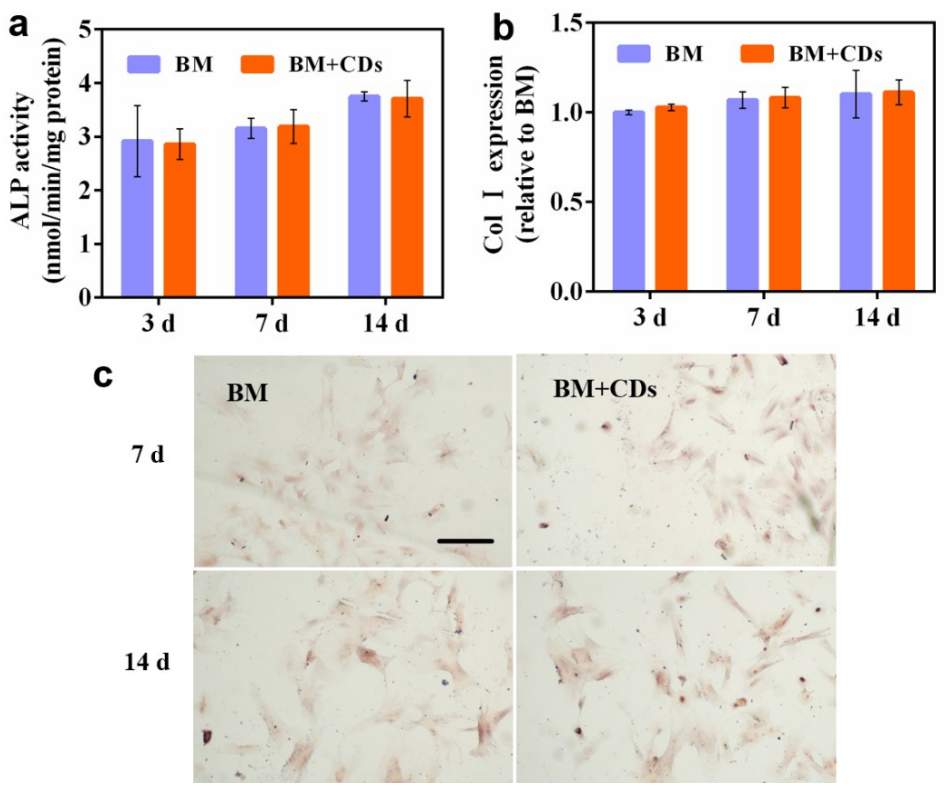

Fig. S12 (a) ALP activity and (b) Col I expression (relative to BM) of rBMSCs incubated in basic mediums (BM) without and with CDs (*conc.*=25 μg/mL) for 3, 7 and 14 days (mean values ± SD), (c) optical microscope images of calcium nodules stained with alizarin red S after rBMSCs cultured in different mediums for 7 and 14 days (scale bar=200 μm).

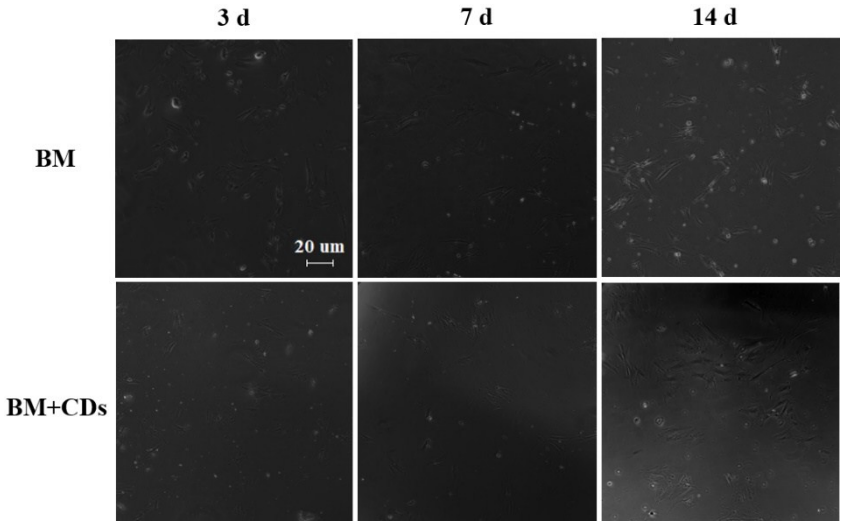

Fig. S13 Bright field images of rBMSCs incubated in basic medium (BM) without and with CDs (*conc.*=25 μg/mL) for 3, 7 and 14 days.

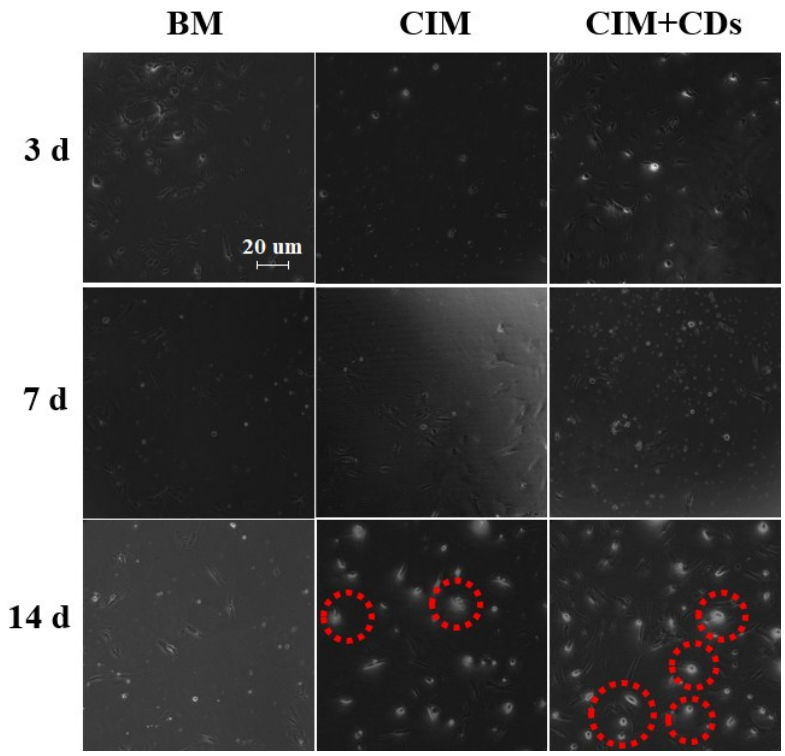

Fig. S14 Bright field images of rBMSCs incubated in different mediums without and with CDs (*conc.*=25 μg/mL) for 3, 7, and 14 days. (red circles showed chondrocytes with spherical morphology).

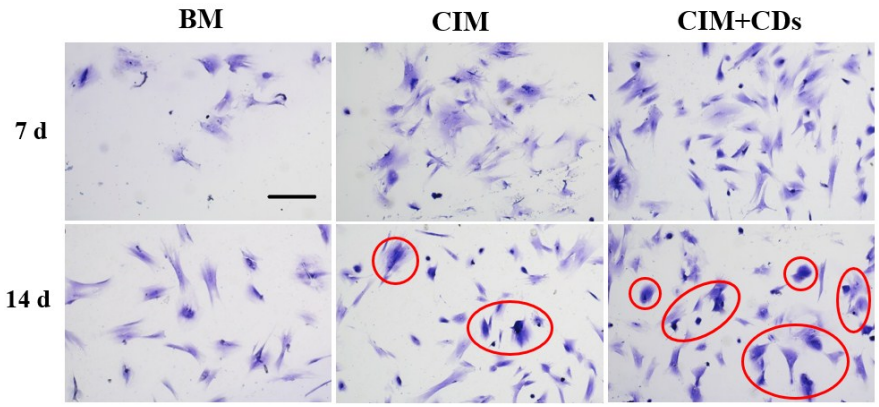

Fig. S15 Optical microscope images of toluidine blue O staining after rBMSCs cultured in different mediums for 7 and 14 d, *conc.* of CDs=25 μg/mL, scale bar = 200 μm.

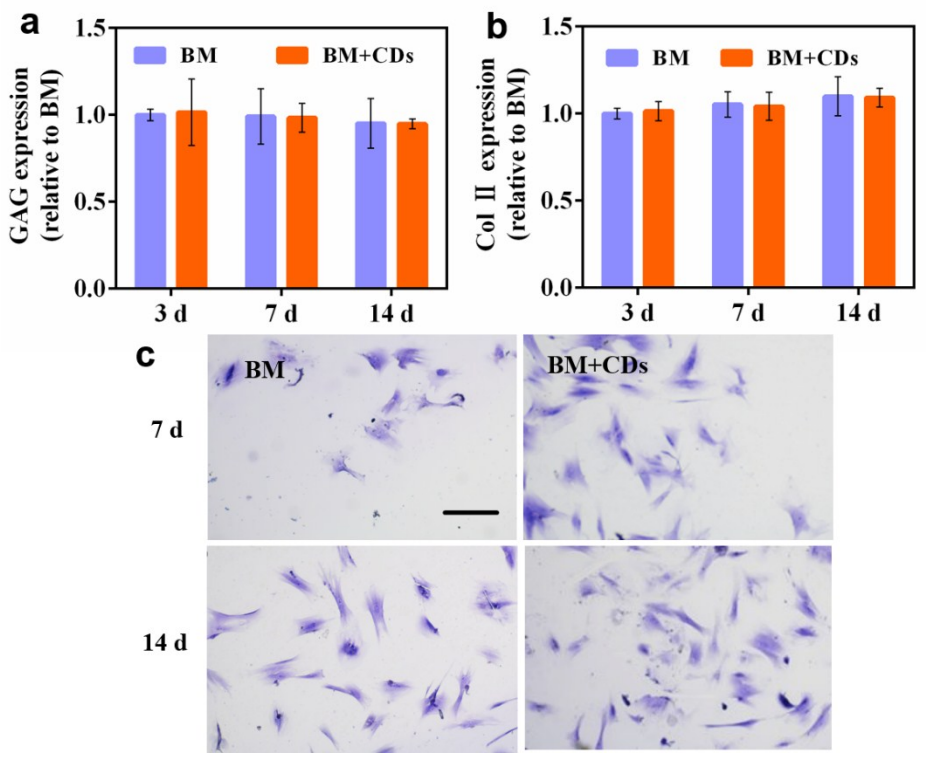

Fig. S16 (a) GAG and (b) Col II expression (relative to BM) of rBMSCs incubated in basic medium (BM) without and with CDs (*conc.*=25 μg/mL) for 3, 7 and 14 days (mean values±SD), (c) optical microscope images of calcium nodules stained with toluidine blue O after rBMSCs cultured in different mediums for 7 and 14 days (scale bar=200 μm).

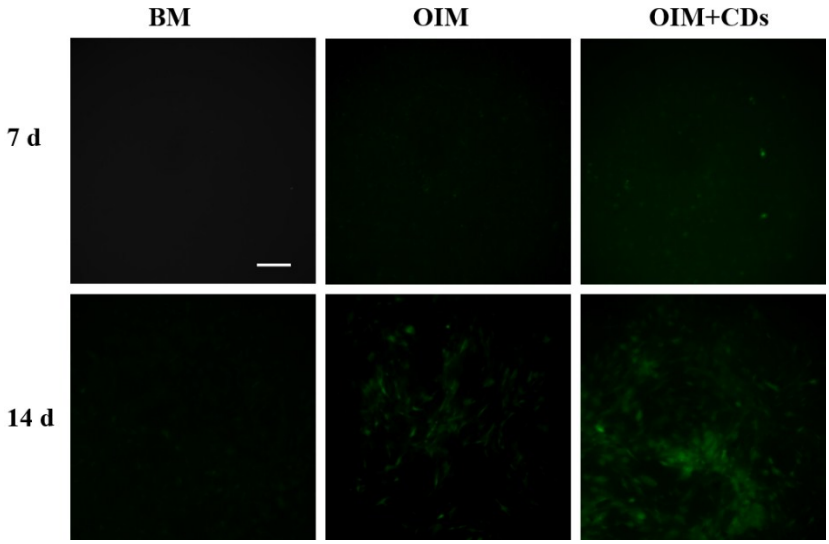

Fig. S17 Intracellular ROS detection of rMSCs cultured in different culture media (BM, OIM and OIM+CDs) for different days (7, and 14 d). DCFH-DA as ROS sensor, scale bar=200 μm.

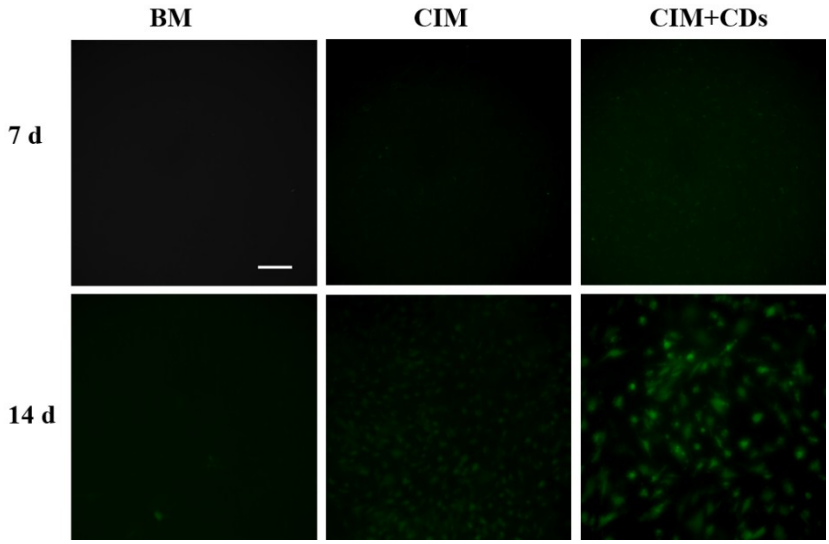

Fig. S18 Intracellular ROS detection of rMSCs cultured in different culture media (BM, CIM and CIM+CDs) for different days (7, and 14 d). DCFH-DA as ROS sensor, scale bar=200 μm.