Supporting Information: Integrating Soft Hydrogel with Nanostructures Reinforces Stem Cell Adhesion and Differentiation

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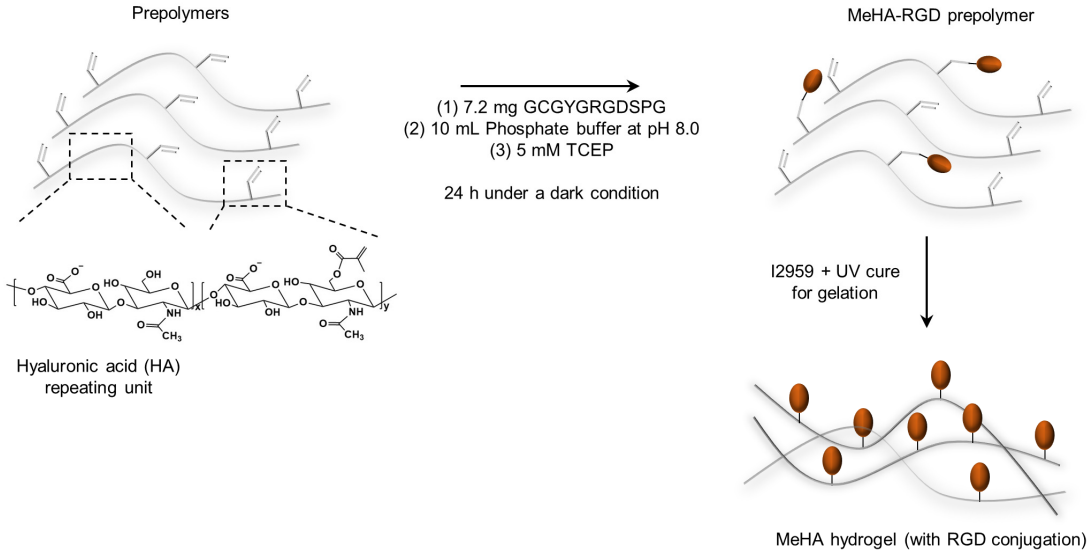

Figure S1. Synthetic route of RGD-coupled methcrylated hyaluronic acid (MeHA) hydrogel.

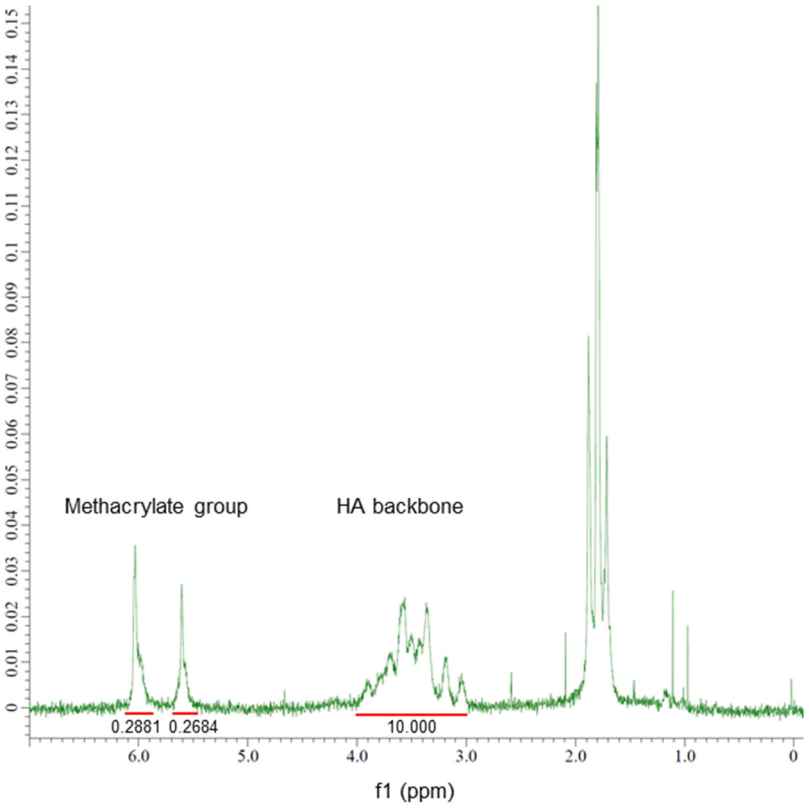

Figure S2. ^{1}H NMR spectra of the methacrylated hyaluronic acid (MeHA). The integration of the spectrum shows that methacrylation degree is ~30%, confirming the successful methacrylation in this study.

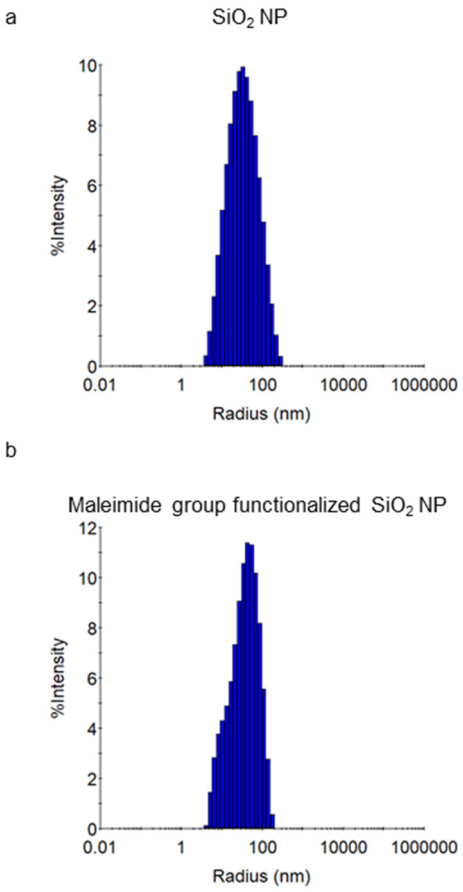

Figure S3. Dynamic light scattering (DLS) characterization for the size of (a) SiO NPs, (b) maleimide group functionalized SiO_2 NPs. The average hydrodynamic measured diameters of (a) and (b) were 31.4 ± 2.5 nm and 39.5 ± 5.4 nm, respectively.

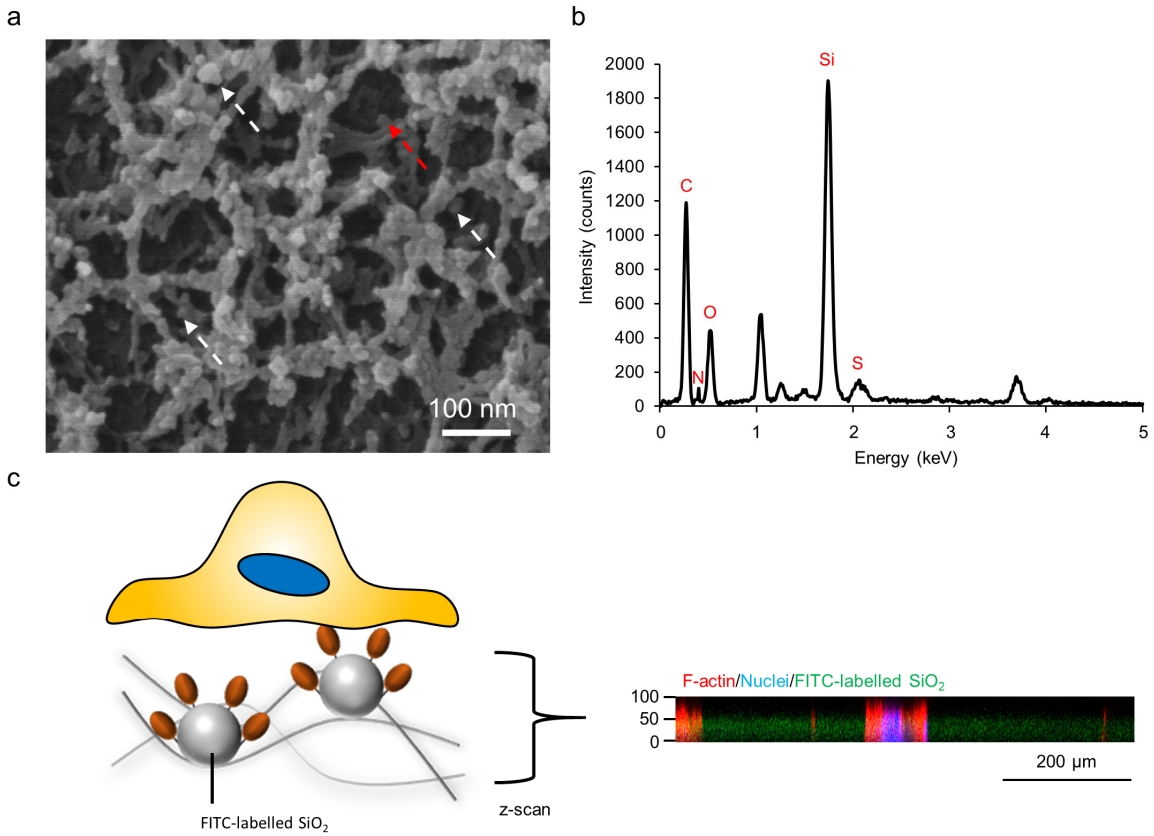

Figure S4. Exploration of immobilized SiO_2 NPs in MeHA hydrogel network. (a) Magnified SEM image of MeHA–SiO2 hydrogel. White arrows indicate the immobilized SiO_2 NPs distributed throughout different layers of MeHA hydrogel. (b) EDS spectrum from red arrow in (a), indicating high content of SiO_2 inside the MeHA network. (c) Cells cultured on MeHA–SiO2 hydrogel where SiO_2 NPs were labelled with FITC. The right panel shows the z-axis stacking images of the FITC-labelled SiO_2 (green) conjugated on MeHA hydrogel, with the presence of cells cultured on the hydrogel (6 h of cell culture). The cells were also stained with F-actin (red) and nuclei (blue).

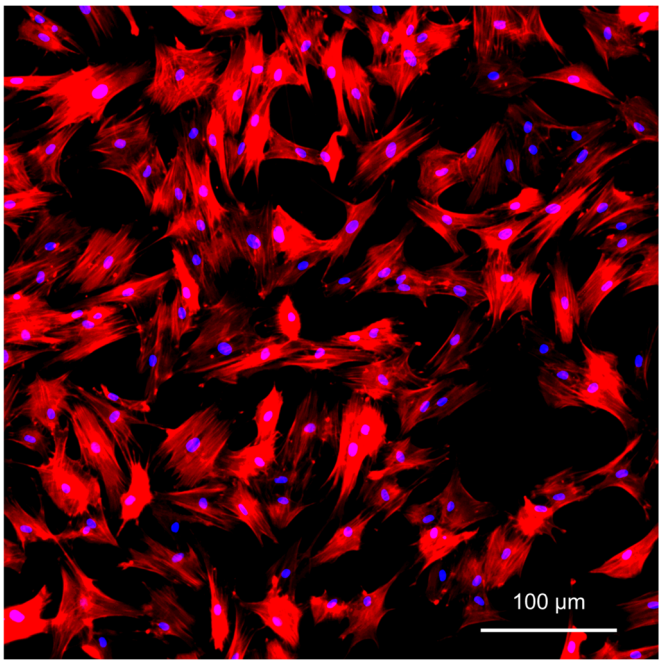

Figure S5. Cells cultured on a glass substrate were considered as a positive control group as the cells spread well on it. The spreading area was 5841 ± 518 µm^2.

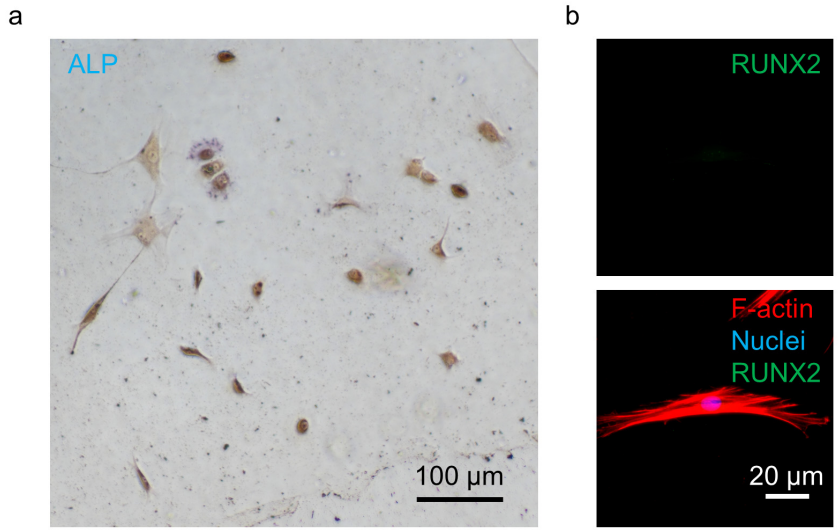

Figure S6. Examination of the expression level of ALP and RUNX2, the early-stage markers of osteogenic differentiation of stem cells, in hMSCs without the osteogenic medium by immunostaining on MeHA hydrogel (with RGD conjugation). (a) ALP staining result. There were only 8.41 ± 2.13 % of positively stained cells for expressing ALP. (b) Immunostaining result RUNX2 (green) merging with F-actin and nuclei staining. The nuclear localization ratio of RUNX2 was 0.37 ± 0.82 in the cells. These results suggest that undifferentiated hMSCs show minimal basal expression of these two markers.