Supplementary figures:



1. The histograms of transfection efficiency.

Figure S1. The transfection efficiency of green fluorescent protein plasmid into hMSCs by flow cytometry.

2. Determination of the appropriate timing of adding serum-containing media in the transfection protocol.

Methods: 5×10^4 cells/well hMSCs were cultured on planar silica (Planar) and NH₂modified silica nanosheets (NS-NH₂) in 24-well tissue culture plates and exposed to the naked GFP plasmid for 12 h. For the 0 h group, cells were seeded in 1 ml of serum-containing medium containing 1 µg GFP plasmid. After 12 h of cell incubation, the medium was changed to 10% serum-containing medium without plasmid. After another 48 h (60 h post cell seeding), cells were washed with PBS, harvested with 0.25% trypsin for 1 min, and centrifuged to remove the supernatant. For the 12 h group, the transfection protocol was the same as that described in the main text. For the 60 h group, cells were seeded in 1 ml of serum-free medium containing 1 µg GFP plasmid. After 12 h of cell incubation, the medium was changed to serum-free medium without plasmid. After another 48 h (60 h post cell seeding), cells were harvested for analysis. For each protocol, the plasmid exposure time remained the same (12 h). The flow cytometry was used to analyze the green fluorescence expression after the plasmid exposure.

Results: The timing of adding serum-containing medium could affect the transfection efficiency and survival rate of hMSCs. Cells on NS-NH₂ had greater transfection efficiency than those on Planar. The cell transfection efficiency on NS-

NH₂ did not differ between the groups of 12 h and 60 h. However, the cell survival rate was significantly lower in the 60 h group. For this reason, we determined in the protocol to change the serum-free medium into serum-containing medium at 12 h in order to obtain the highest transfection efficiency and cell survival rate.



Figure S2. (A) The transfection efficiency and (B) survival rate of hMSCs cultured on planar silica (Planar) and NH₂-modified silica nanosheets (NS-NH₂). All cells were analyzed at 60 h post seeding. ***, p < 0.001.