Supporting Information

Oh *et al.* 10.1073/pnas.0813200106

SI Materials and Methods

Cell Counting and Viability Test. A coulter particle counter (Beckman Coulter; Z-1 model) was used to count the hMSCs for maintenance and experimental plating densities. To measure live cell elongation at various time points, a fluorescein diacetate (FDA; Sigma) staining was conducted. Cells were plated onto the specimen at a density of 1×10^4 cells per mL. At 2 and 24 h after plating, the cells on the substrate of the specimen were washed by 1× PBS, stained by FDA in PBS (10 μL/10 mL), incubated for ≈30 s, and washed once more.

Immunofluorescence of Fibronectin. After 2 h of incubation in cell culture media or control H_2O with out the presence of cells, Ti and TiO_2 nanotube substrates were fixed in 4% paraformaldehyde in 1× PBS for 15 min at room temperature. Once fixed, substrates were washed twice with 1× wash buffer (1× PBS containing 0.05% Tween-20). Then the samples were incubated for 1 h at room temperature in 5% gelatin (Sigma, from fish skin)/1× PBS followed by the addition of primary anti-BSA antibody (1:100, 2A3E6, Santa Cruz Biotechnology)/anti-bovine serum fibronectin antibody (1:1,000; Primary antibody for staining fibronectin was R457 rabbit polyclonal anti-rat antiserum against the amino-terminal 70-kDa fragment of fibronectin). The samples were incubated overnight at 4 °C. After incubation, substrates were washed three times for 5 min each wash with 1× wash buffer. Goat anti-mouse IgG-FITC (1:1,000, Santa Cruz Biotechnology) and goat anti-rabbit IgG Alexa Fluor 594 (1:1,000, Invitrogen) in 1× PBS was added for double staining and the substrates were incubated again for 1 h at room temperature. After incubation, substrates were washed 3 times for 5 min each wash with 1× wash buffer. The samples were then inverted onto coverslips, mounted, visualized, and photographed by an epifluorescence microscope (DM IRB, Leica Microsystems).

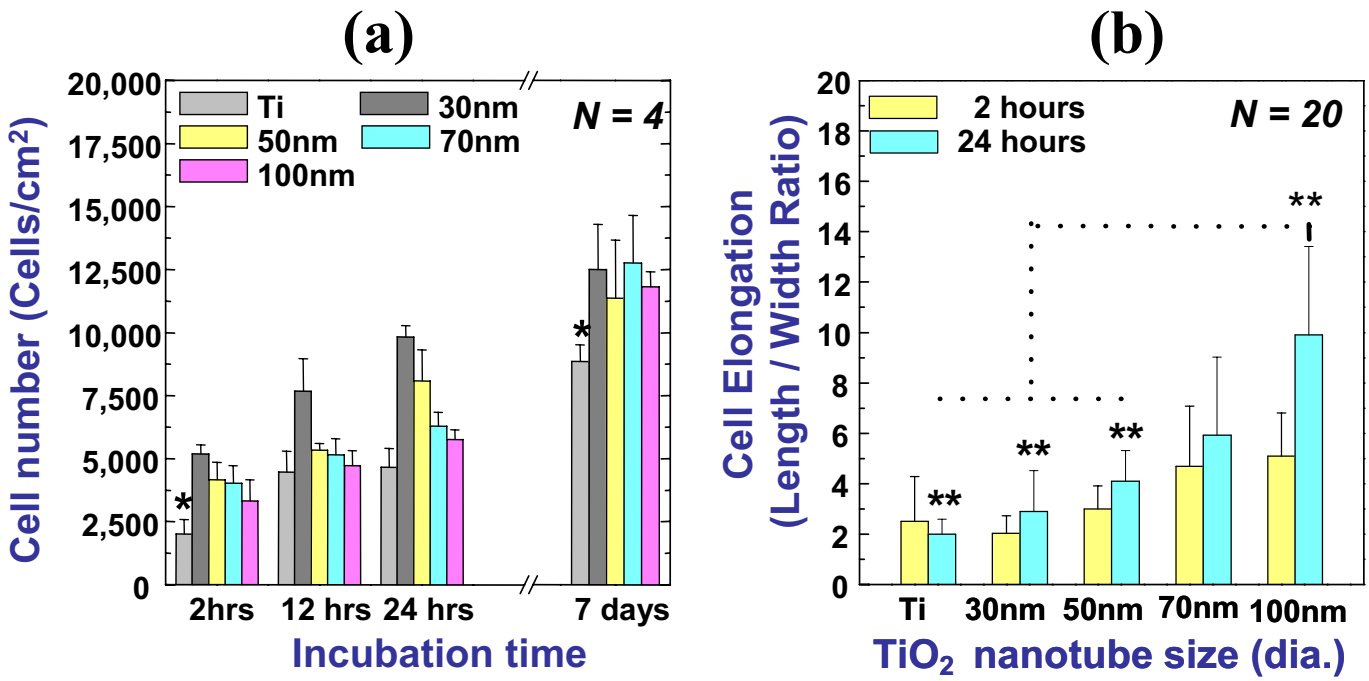

Fig. S1. hMSC behavior on Ti and nanotube substrates. (*a*) Cell number vs. incubation time for various TiO_2 nanotube diameters. (*b*) Cell elongation vs. TiO_2 nanotube diameter at 2 and 24 h. (*c*) Schematic illustration of the overall trend of nano cue effect on hMSC control, for example, after 24 h culture. *, Significant statistical difference between Ti vs. TiO_2 nanotubes ($P < 0.01$); **, significant difference between Ti, 30-, 50-nm nanotube vs. 100-nm nanotube ($P < 0.01$).

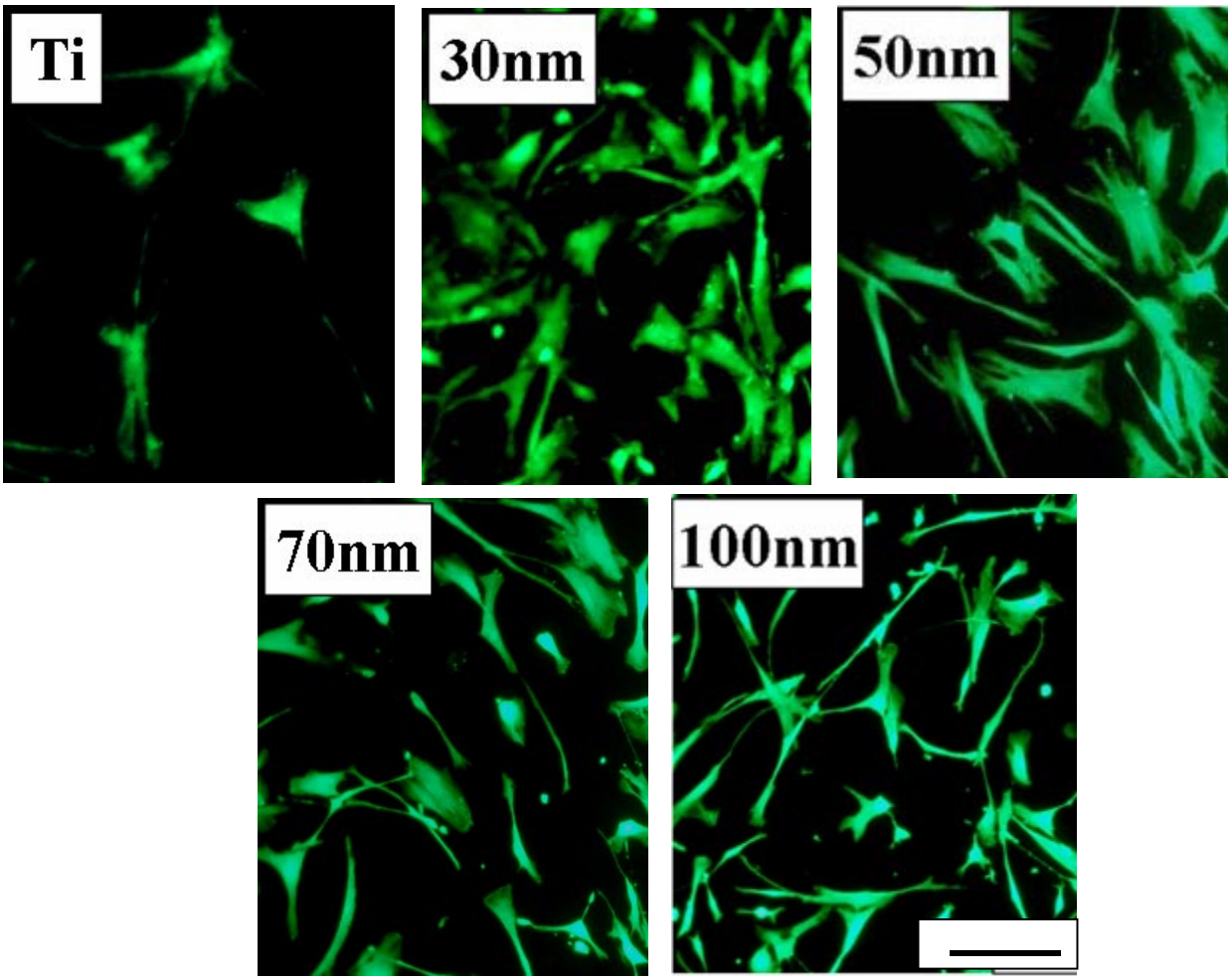

Fig. S2. Fluorescein diacetate (FDA) images of human mesenchymal stem cells (hMSCs) on flat Ti and 30-, 50-, 70-, and 100-nm diameter TiO_2 nanotube surfaces after 24 h of culture. (Scale bar, 100 μm.)

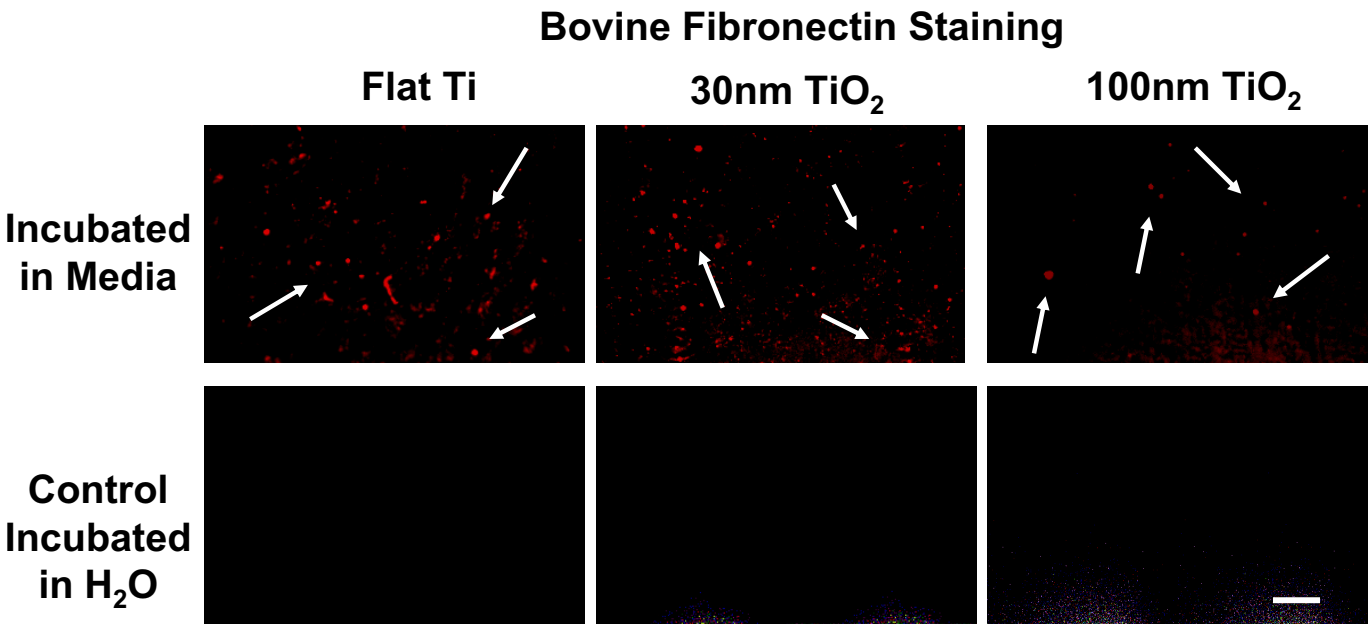

Fig. S3. Bovine fibronectin immunofluorescent staining (red) was performed on flat Ti, 30-nm, and 100-nm TiO_2 surfaces to identify the protein particles on the surface. Fibronectin particles were detected on all surfaces that were incubated in media for 2 h. No fibronectin was detected on control surfaces incubated for 2 h in H_2O. (Scale bar, 20 μm.)