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Supporting Information

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Carbon Nanotube Monolayer Cues for Osteogenesis of Mesenchymal Stem Cells

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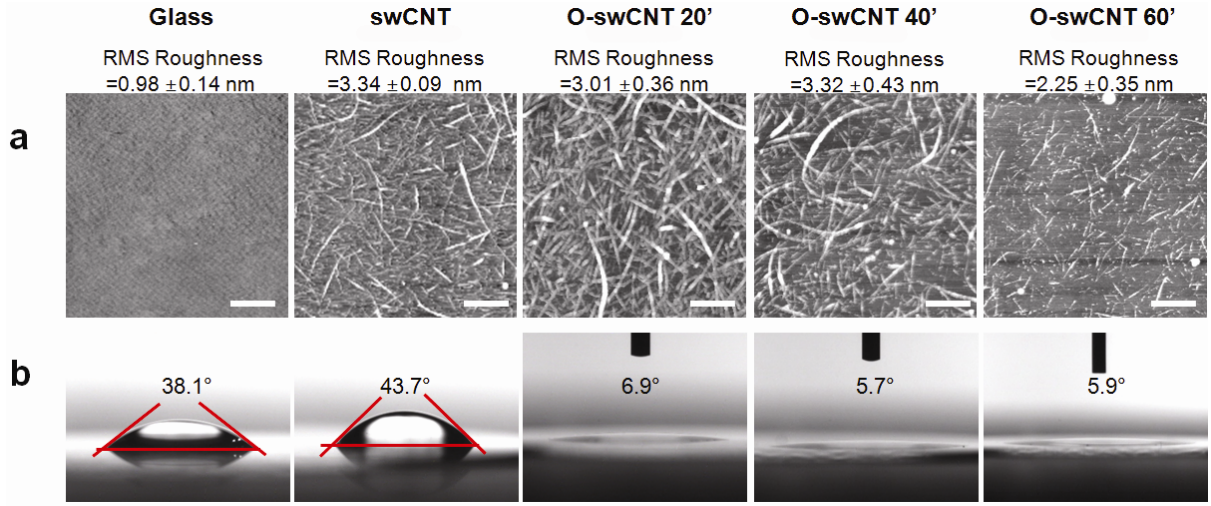


Figure S1. Surface property of swCNT coated substrates. (a) The AFM images of swCNT coated glass substrates with and without oxygen plasma treatment. Bare glass surface was used as a control. The roughness of swCNT monolayer was 3.34 ± 0.09 nm, and it remains almost constant with the oxygen plasma treatment up to 40 seconds' exposure. The longer exposure blew off swCNTs from the glass substrate. Scale bars are 1 μm. (b) The swCNT coating on the glass slightly increased its hydrophobicity, but oxygen plasma treatment for 20 seconds abruptly decreased the contact angle of distilled water.

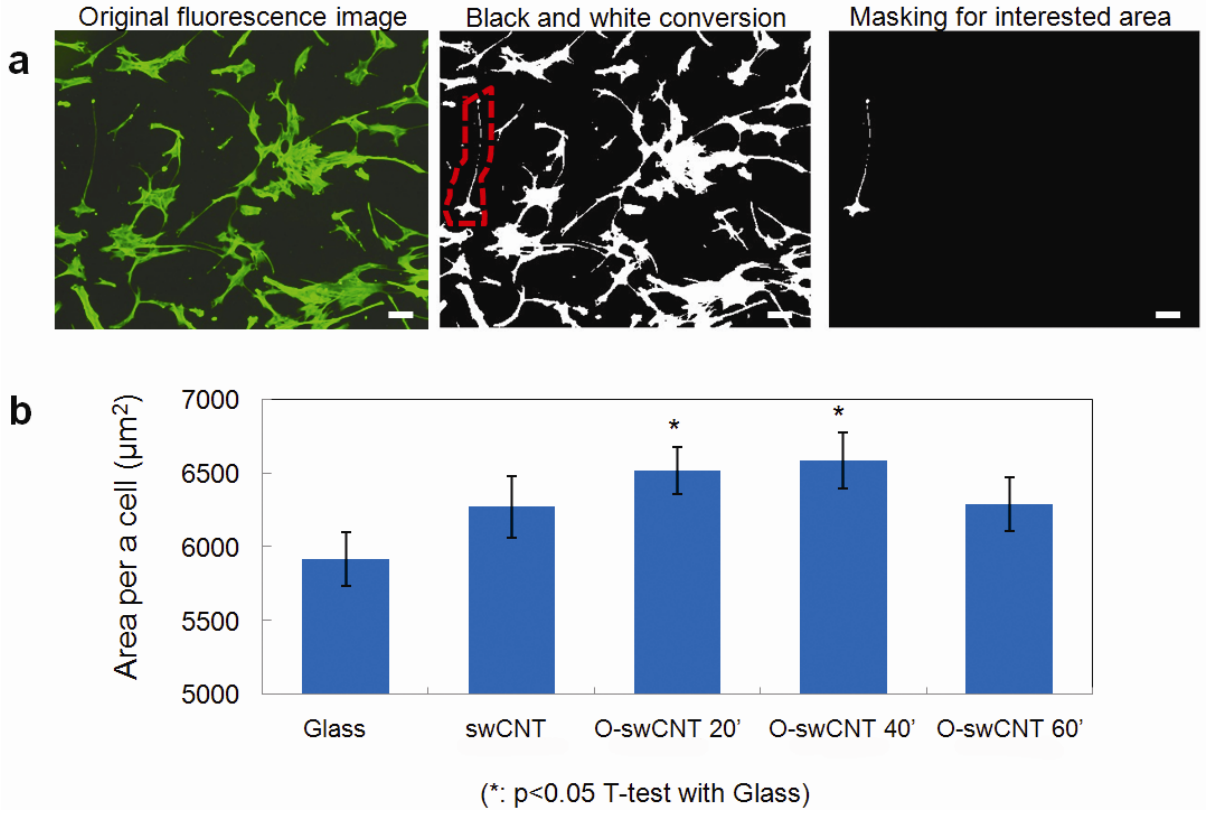


Figure S2. Analysis of the area per a cell on different substrates. (a) To measure the cell adhesion area, cells were stained with FITC-phalloidin to reveal their actin filaments. The green fluorescence intensity was converted to the values of '0' or '1' (black and white) by subtracting the averaged background intensity, and each cell was selected using mask as shown in the pictures. The number of pixels whose value is '1' was counted, and then the number of pixel was used to calculate the area of each cell. Scale bars are 100 μm. (b) Graph showing the area per a cell on different substrates. 200 cells per each substrate were analyzed to get mean values. The graph shows that the area per a cell increased on swCNT monolayer compared with that of glass substrates. The value is maximized with 20 - 40 seconds' oxygen plasma exposure which might induce optimal conditions for cell adhesion.

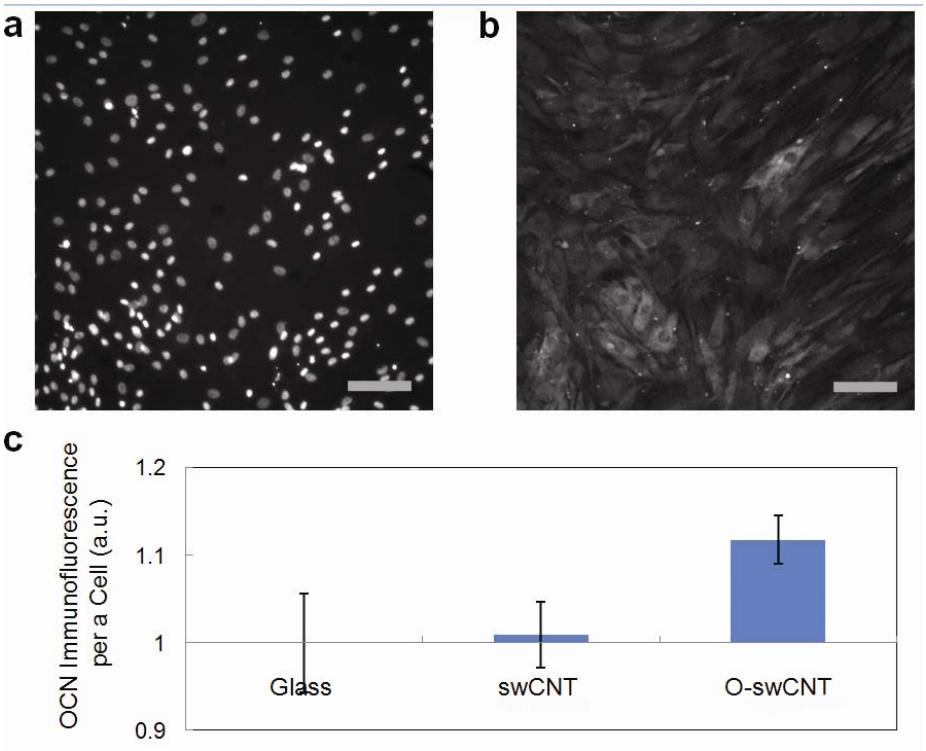
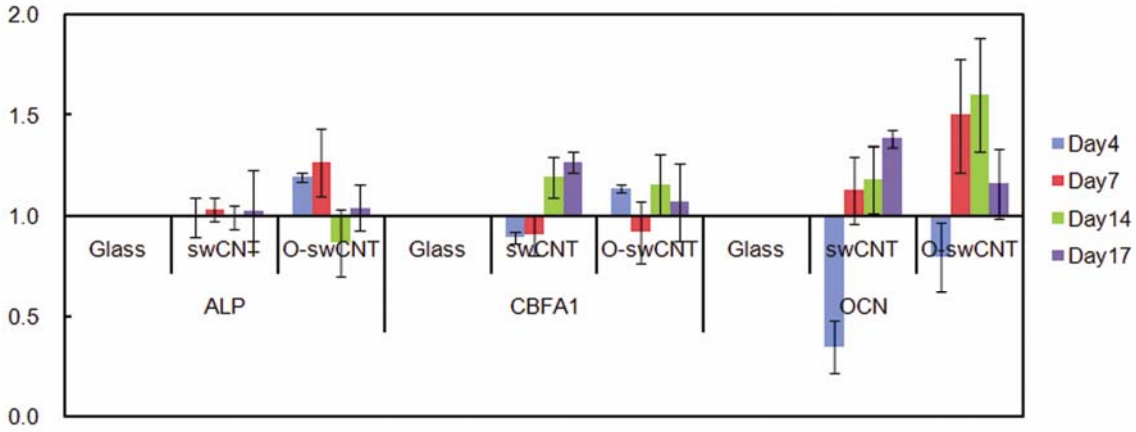


Figure S3. Quantification of OCN immunofluorescence. (a) Fluorescence image of DAPI stained nuclei. The number of nuclei was counted using MATLAB software to estimate the number of cells in the image. (b) OCN immunofluorescence image. Scale bars are 100 μm. (c) Graph showing OCN immunofluorescence intensity per a cell on different substrates. The intensity value in y-axis was normalized to that from the glass substrate. The OCN immunofluorescence intensity per a cell was calculated by dividing the sum of OCN immunofluorescence intensity from the whole image (b) by the number of the cells (a). hMSCs grown on O-swCNT substrate exhibited enhanced OCN proteins compared with those on glass substrate.

a



b

Marker Name	Sequence	size	Acc. No.
Beta-actin (ACTB)	F; 5'-GGCATCCTCACCCTGAAGTA	82	NM_001101.3
	R; 5'-AGGTGTGGTGCCAGATTTTC		
Alkaline phosphatase (ALP)	F; 5'-GGAACTCCTGACCCTTGACC	86	NM_000478.3
	R; 5'-TCCTGTTCAGCTCGTACTGC		
Osteocalcin (OCN)	F; 5'-ACCCTTCTTTCCTCTTCCCCTT	87	NM_199173.3
	R; 5'-TATTTGGGAGCAGCTGGGATGA		
Core binding factor A1 (RUNX2 or CBFA1)	F; 5'-TGACACCACCAGGCCAATCC	123	NM_001015051
	R; 5'-ACAAGGGGGCCGTTAGGGTT		

Figure S4. mRNA analysis using qPCR on swCNT substrates. (a) qPCR data of hMSCs on glass substrates, swCNT monolayer substrates and O- swCNT monolayer substrates from day 4 to day 17. Osteogenic markers -alkaline phosphatase (ALP), core binding factor A1 (CBFA1), and osteocalcin (OCN) mRNA were quantified. CBFA1 and OCN were upregulated with time on both swCNT substrates compared with the glass substrate, while ALP mRNA was enhanced only in early stage on O-swCNTs. (b) Sequence, product size and accession number in NIH website of primers were tabulated.

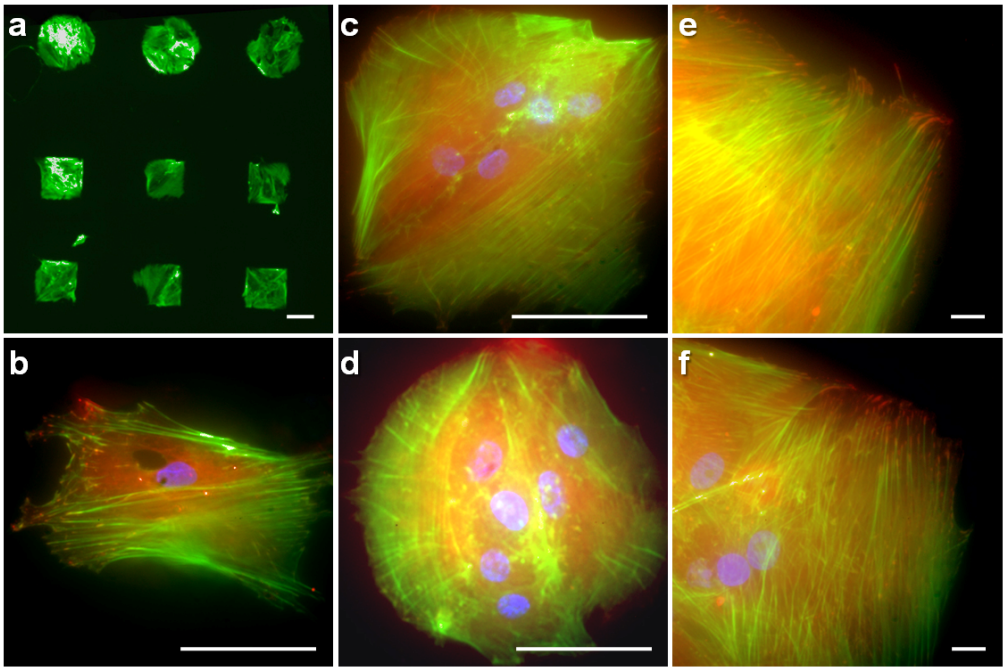


Figure S5. Fluorescence images showing cytoskeletons of hMSCs on swCNT patterns. The hMSCs were confined in various shape swCNT monolayer patterns. Actin filaments, focal adhesion protein (vinculin), and nuclei were stained by phalloidin (green), fluorescence-labeled antibody (red), and DAPI (blue), respectively. (a) hMSCs confined in 200 μm square and 300 μm diameter circular patterns. Scale bar is 200 μm. (b) A single cell occupying a 200- μm -size square pattern. The stretched actin filaments were attached to the boundary of square using focal adhesion proteins. Scale bar is 100 μm. (c) hMSCs aligned along diagonal direction in a square pattern. Nuclei were placed close at the center, and cytoplasm surrounded it along the boundary. Scale bar is 100 μm. (d) hMSCs on a circular pattern. The polarity of the cell nucleus and cytoplasm in the circle pattern was similar to those on the square patterns. Scale bar is 100 μm. (e-f) High resolution images showing the focal adhesion (red) on the boundary of a square pattern. Scale bars are 10 μm.