Supplementary Information

Neuron-like differentiation of mesenchymal stem cells on silicon nanowires

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Figure captions

Fig. S1 Behaviors of hMSCs on etched SiNWs. **(**a) Morphological changes of various cells on SiNWs. Fluorescence images of HeLa cells, hMSCs, and HUVECs cultured on either Si wafers or long SiNWs were obtained by CFDA staining. For HUVECs, the Si wafer was coated with FN for proper adhesion. Scale bar, 50 μm. (b) Cell morphology changes on various surfaces. Fluorescence images of hMSCs on various surfaces at day 3 were obtained by CFDA staining. (c) Assessment of cell mobility. A schematic of the experimental procedure is presented (top). A HUVEC solution (10 μL) was dropped on various surfaces and incubated for 1 h until cells were attached to the surface. Cell-attached Si wafers or SiNWs were then transferred into a culture plate with the appropriate media and incubated for 1 day. For comparison, the tube formation of HUVECs on a matrigel-coated plate was included. Fluorescence images of HUVECs on various surfaces were obtained by CFDA staining (bottom). Scale bar, 100 μm. (d) Gene expression analysis of hMSCs. Expression levels of neuronal markers, TUBB3, and NEGR1 at day 3 on various surfaces (top). Each mRNA level was normalized to GAPDH, and the mRNA level of hMSCs on the culture plate was set to 1 for comparison. Expression levels of osteogenic markers, RUNX2 and COL1A1 at day 3 on various surfaces in the presence of osteogenic supplements (Pt-3002, Lonza, USA) (bottom). PL, plate; LN, long SiNWs; N, normal medium; D, medium with osteogenic supplements. Each mRNA level was normalized to GAPDH, and the mRNA level of hMSCs on the culture plate without osteogenic supplements was set to 1 for comparison. Detailed information on the SiNWs is described elsewhere.[1]

Fig. S2 Live/dead cell assays of hMSCs on Si wafers and long SiNWs at day 3. Scale bar, 100 μm. The relative ratio of PI stained cells (%) was calculated. The bar represents the mean ± S.D. (*$p<0.05$)

Fig. S1

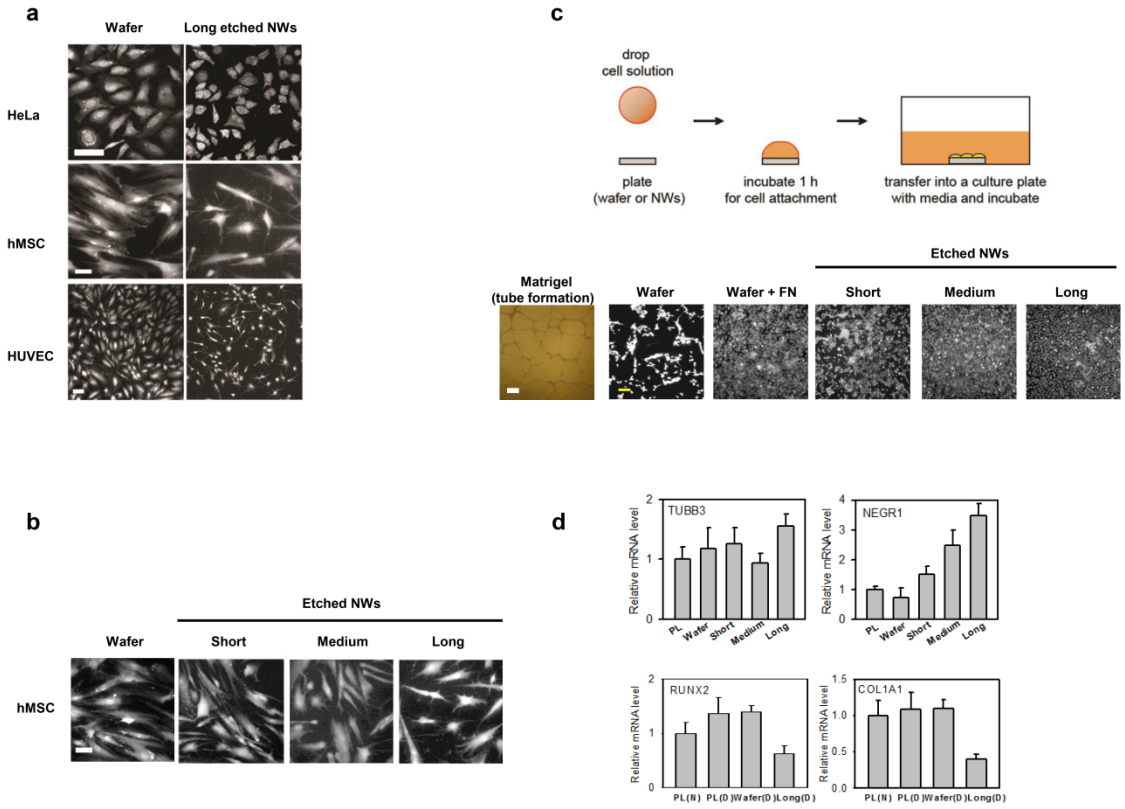
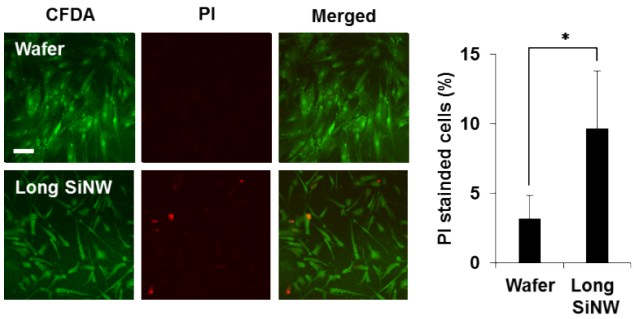

a
Wafer
Long etched NWs
HeLa
hMSC
HUVEC
c
drop
cell solution
plate
(wafer or NWs)
incubate 1 h
for cell attachment
transfer into a culture plate
with media and incubate
Etched NWs
Matrigel
(tube formation)
Wafer
Wafer + FN
Short
Medium
Long
b
Etched NWs
Wafer
Short
Medium
Long
hMSC
d
TUBB3
NEGR1
RUNX2
COL1A1
Relative mRNA level
PL
Wafer
Short
Medium
Long
PL(N)
PL(D)
Wafer(D)
Long(D)

Fig. S2


CFDA
PI
Merged
Wafer
Long SiNW
PI stainded cells (%)
15
10
5
0
*
Wafer
Long SiNW

Movies

Movies S1 and S2. Time-lapse imaging of EGFP-expressing hMSCs on Si wafers. Images were taken every 10 min for 10 h (60 frames in total). Images are presented as 10 frames/sec (6000X faster).

Movies S3 and S4. Time-lapse imaging of EGFP-expressing hMSCs on SiNWs. Images were taken every 10 min for 10 h (60 frames in total). Images are presented as 10 frames/sec (6000X faster).

Table S1. Primer sequences for real-time PCR

Target	Primer sequences (5′ to 3′)
NES forward	CTG CTA CCC TTG AGA CAC CTG
NES reverse	GGG CTC TGA TCT CTG CAT CTA C
NEUROD forward	GTC TCC TTC GTT CAG ACG CT
NEUROD reverse	AAA GTC CGA GGA TTG AGT TGC
TUBB3 forward	GCG AGA TGT ACG AAG ACG AC
TUBB3 reverse	TTT AGA CAC TGC TGG CTT CG
NEGR1 forward	CAG ACT CAA CAT ACA CCC AGA AC
NEGR1 reverse	AAA CAA GTA AGA GTG ACG TTG GT
RUNX2 forward	CTA CCA CCC CGC TGT CTT C
RUNX2 reverse	CAG AGG TGG CAG TGT CAT CA
COL1A1 forward	ATG TTC AGC TTT GTG GAC CTC
COL1 A1 reverse	CTG TAC GCA GGT GAT TGG TG
GAPDH forward	GGA AGG TGA AGG TCG GAG TCA
GAPDH reverse	GTC ATT GAT GGC AAC AAT ATC CAC T

References

1. S. Y. Kim and E. G. Yang, *Nanotechnology*, 2013, **24**, 455704.