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# Supplementary Information

# for

# Facile oxidation of superaligned carbon nanotube films for primary cell culture and genetic engineering

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**Materials and Methods.**

**Characterization of CNT films.** For SEM images, both untreated or oxidized CNT films were cut by laser and placed onto a $1\times1$ $cm^2$ glass coverslide that was pre-treated with 0.1% (w/v) PEI. Samples were completely dried and characterized using a FEI Sirion SEM (electron beam = 10 kv). For analysis of Raman spectroscopy, an area of $4\times4$ $cm^2$ CNT film was individually placed onto a square Teflon frame (shown in Figure 1, outer length = 40 mm, inner length = 30 mm, with a frame length of 5 mm), and dried completely. All samples were then investigated by Raman spectra, using a Renishaw spectrometer (1800 gr/mm grating mode) with a laser excitation at 514.5 nm. Raman shift was scanned from 400 to 2800 $cm^{-1}$. The laser was focused to a 1-2 μm spot size on the samples and the power was set to less than 2 mW. 3 different spots on one film were randomly measured, and the characteristic peak intensities of D- and G-line were recorded to calculate D/G ratio, showing the quality of CNT films. Such prepared samples were further used for contact angle measurements. Contact angle values were measured at room temperatures, using an optical contact angle measuring device (OCA-20, DataPhysics Instruments GmbH, Filderstadt, Germany). In each measurement, a water droplet of 2 μL was dispensed onto the surface of CNT films. Water contact angles of at least 3 different spots on one CNT film were acquired and averaged. Fourier Transform Infrared Spectra (FT-IR) were next acquired for CNT films, collected on a Bruker IFS 66 v/s spectrometer at 2-$cm^{-1}$ resolution. For XPS measurements, an area of $1\times1$ $cm^2$ CNT film was placed

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onto silicon wafer, wetted by ethanol and left completely dry. XPS measurements were then performed in a PHI-5300ESCA Perkin-Elmer spectrometer, using monochromatic Mg Kα (1253.6 eV) radiation at 14 kV and 250 W. Element analyses were conducted for C, O, and N elements. CNT films after the same preparation were subject to measurements of electric resistance in a four-wire mode.

**Cell isolation and culture.** The use of experimental animal was reviewed and approved by School of Medicine, Tsinghua University, China. To isolate MEF cells, a 13.5-day pregnant mouse was sacrificed by cervical dislocation. Abdomen was liberally covered with 70% alcohol, then cut through skin and peritoneum to expose the uterine horns, followed by removal of the uterine horns. Skin tissues were placed in a Petri dish containing PBS (w/o $Ca^{2+}$, $Mg^{2+}$). Embryos were removed from the embryonic sac and then dissected out and the placenta and membranes were discarded. The embryos were decapitated and eviscerated, and the remaining carcases were washed 3 times with PBS (w/o $Ca^{2+}$, $Mg^{2+}$). Carcasses were put in a clean Petri dish and then minced with a scalpel blade, followed by the addition of 2ml trypsin: EDTA and incubation at 37°C for 10-20 minutes. 5ml Dulbecco's modified eagle medium (DMEM)/ fetal calf serum (FCS) were then added and the solution was transferred to a 15-ml centrifuge tube, aspirated vigorously. After the large chunks settled due to gravity, the supernatant was taken to a T75 flask and added with another 15 ml DMEM/FCS, kept in the 37°C incubator over night. At the next day, fresh media were added to remove floating cellular debris. Cells were grown until around 90% confluent

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(counted as the first generation), and then stored at -80 $^0$C. For MEF culturing, cells were maintained at 37°C with 5% $CO_2$ in DMEM (with high glucose, 10% FCS, 1% glutaMAX, 0.5% penicillin/streptomycin, and 0.1% gentamicin).

Cardiomyocytes were isolated from 1- to 2-day-old Sprague-Dawley rats. Briefly, hearts from neonatal rats were rapidly extracted, excised and washed in ice-cold PBS (containing 50 unit/ml heparin) to remove blood and debris. Then the hearts were gently minced by repetitive scissoring and digested by a mixture of 0.04% trypsin and 0.03% collagenase II (in a balanced salt solution containing 137 mM NaCl, 4 mM KCl, 4.2 mM $NaHCO_3$, and 5 mM glucose, adjusted by diluted HCl drop-wise to pH = 7.68) to release single cells. The obtained cells were suspended in DMEM high glucose growth medium, containing 10% FCS for deactivation of proteolytic enzymes). Since the main content of isolated cells included cardiomyocytes and cardiac fibroblasts, the next differential attachment was applied to separate the cells. That is, after 2 hours cell attachment, the supernatant (mainly NRCs) was taken for centrifugation and cell pellet was then collected to be further re-suspend in above DMEM media with 3.1 mg/100ml bromodeoxyuridine (5-bromo-2'-deoxyuridine, BrdU) for elimination of non-muscle cell contamination, plus 0.5% penicillin/streptomycin, 2 mM L-glutamine and 3 mM sodium pyruvate.

**Preparation for SEM images of cells.** For SEM analyses of cell growth on various substrates, cell media were carefully removed and cells on the films were washed three times by PBS, followed by fixation of 2.5% glutaraldehyde (25% glutaraldehyde

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diluted 10 times in 1×PBS) for 3 hours. The films were then washed by PBS for three times, and dehydrated in 30%, 50%, 70%, 80%, 90% (v/v) ethanol solutions, respectively, 5 min for each dehydration. Lastly, films were soaked in 100% ethanol for three times, 5 min each time. When completely dried under air flow, films were coated by 10-nm gold (Hitachi E-1010 Ion Sputter), ready for SEM observation.

**Fluorescence imaging and videoing.** For living cell imaging of MEF, cells were cultured and washed by PBS solution. Hoechst dyes to indicate the cell nuclei (1:800, blue), and PI to stain the dead cells (1:1000, red), both purchased from Dojindo Laboratory, were added into modified DMEM (without addition of serum and phenol red) and incubated with cells at 37°C for 30 min. Samples were washed intensively by PBS solution and observed under fluorescence microscopy (Olympus Q-imaging RETIGA 2000R). For living NRC imaging and videoing, cells were washed by modified DMEM and stained with Fluo-4 AM (1:250, from Dojindo Laboratory) at 37°C for 40 min to visualize cellular $Ca^{2+}$ flux pattern.

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Table S1. XPS measurements were performed in a PHI-5300ESCA Perkin-Elmer spectrometer, using monochromatic Mg Kα (1253.6 eV) radiation at 14 kV and 250 W. Element analyses were conducted for C, O, and N elements. O/C and N/C stand for mole ratios of oxygen and nitrogen to carbon, respectively.

| **Samples** | **$C_{1s}$, %** | **$O_{1s}$, %** | **$N_{1s}$, %** | **O/C** | **N/C** |
|---|---|---|---|---|---|
| **Untreated CNT film** | **93.5** | **6.5** | **0.06** | **0.07** | **<0.001** |
| **Oxidized CNT film** | **79.0** | **19.6** | **1.5** | **0.23** | **0.02** |

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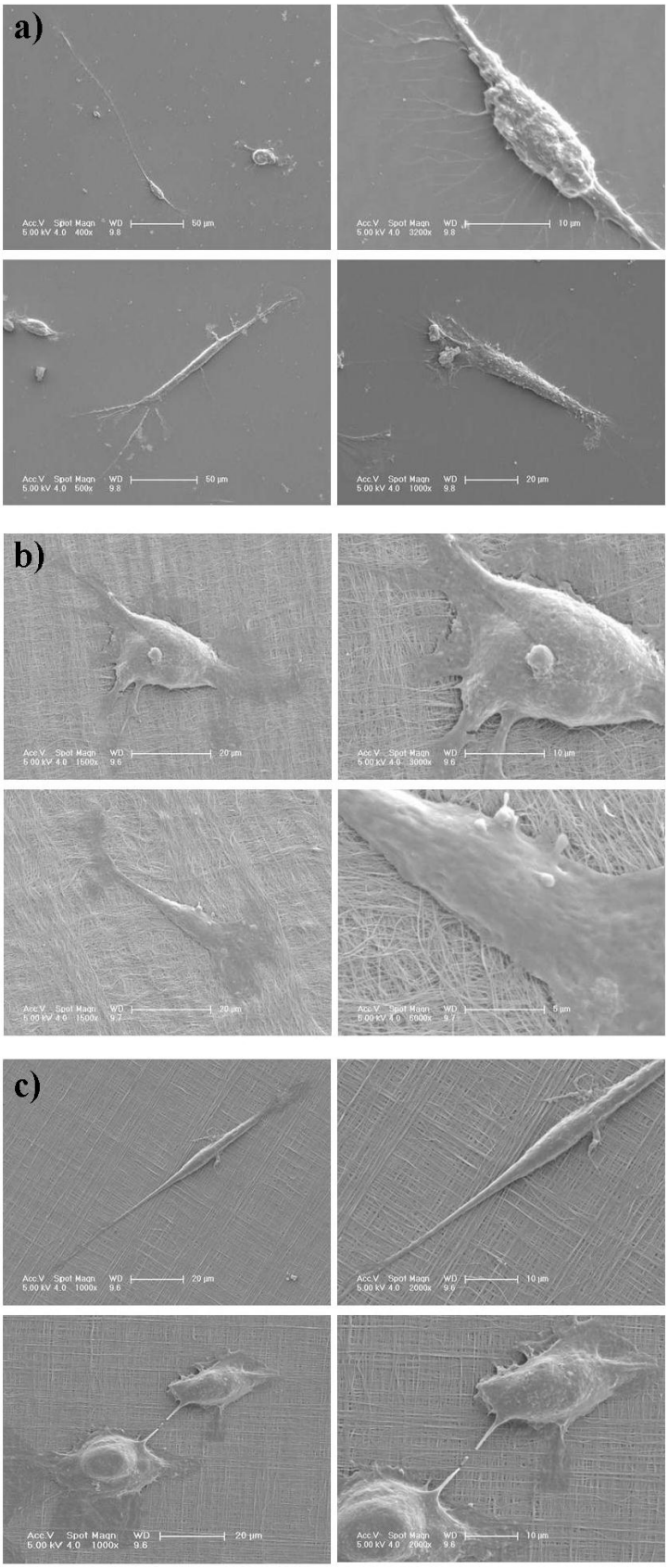


Figure S1. SEM images of MEFs when grown on a) cell dish; b) untreated CNT film; c) oxidized CNT film.

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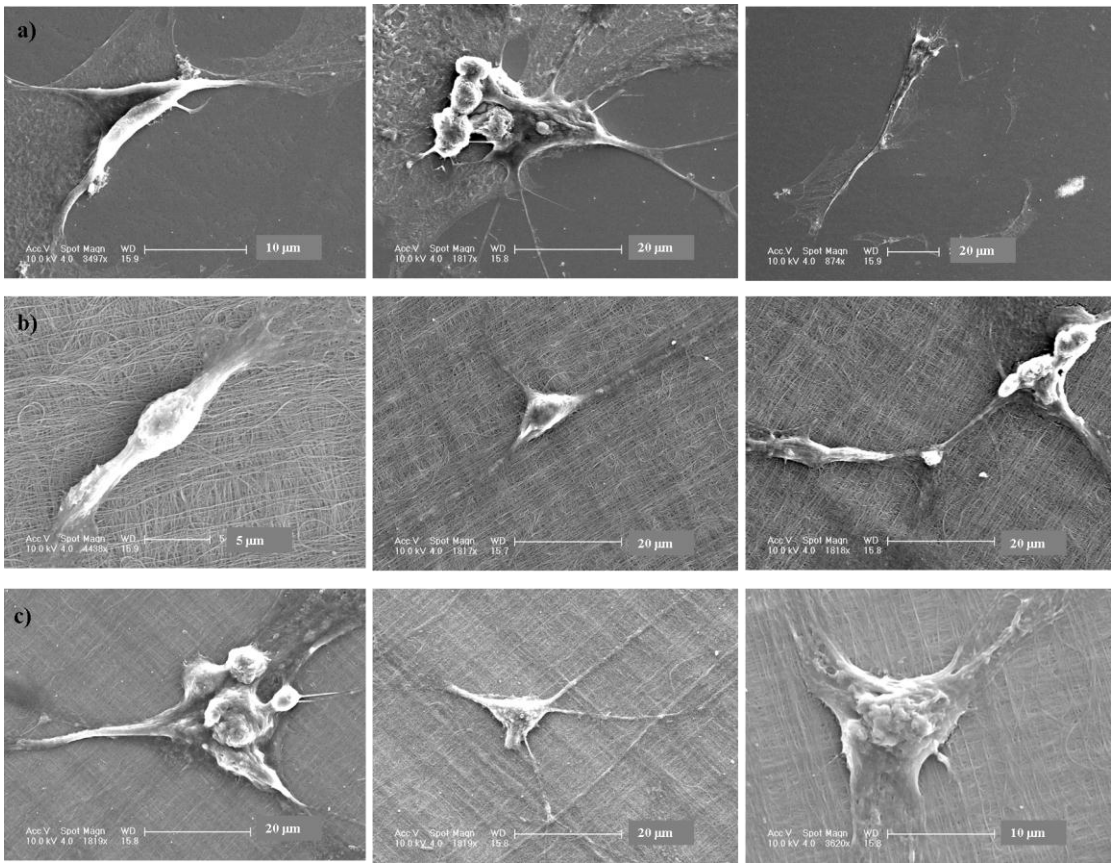


Figure S2. SEM images of NRCs when grown on a) cell dish; b) untreated CNT film; c) oxidized CNT film. Scale bars are as indicated.

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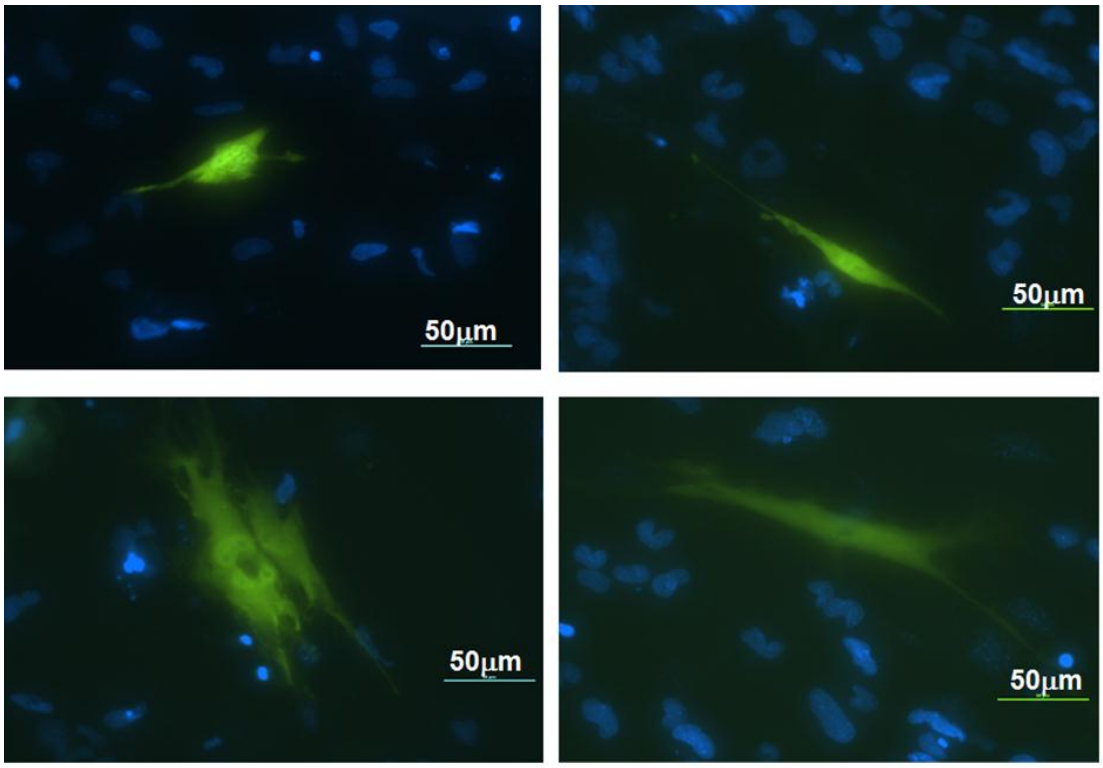


Figure S3. MEFs, stained by Hoechst blue in nuclei, were grown in oxidized SACNT films, while GFP DNA plasmids were transfected into cells directly. Successfully transfected cells were indicated in green.

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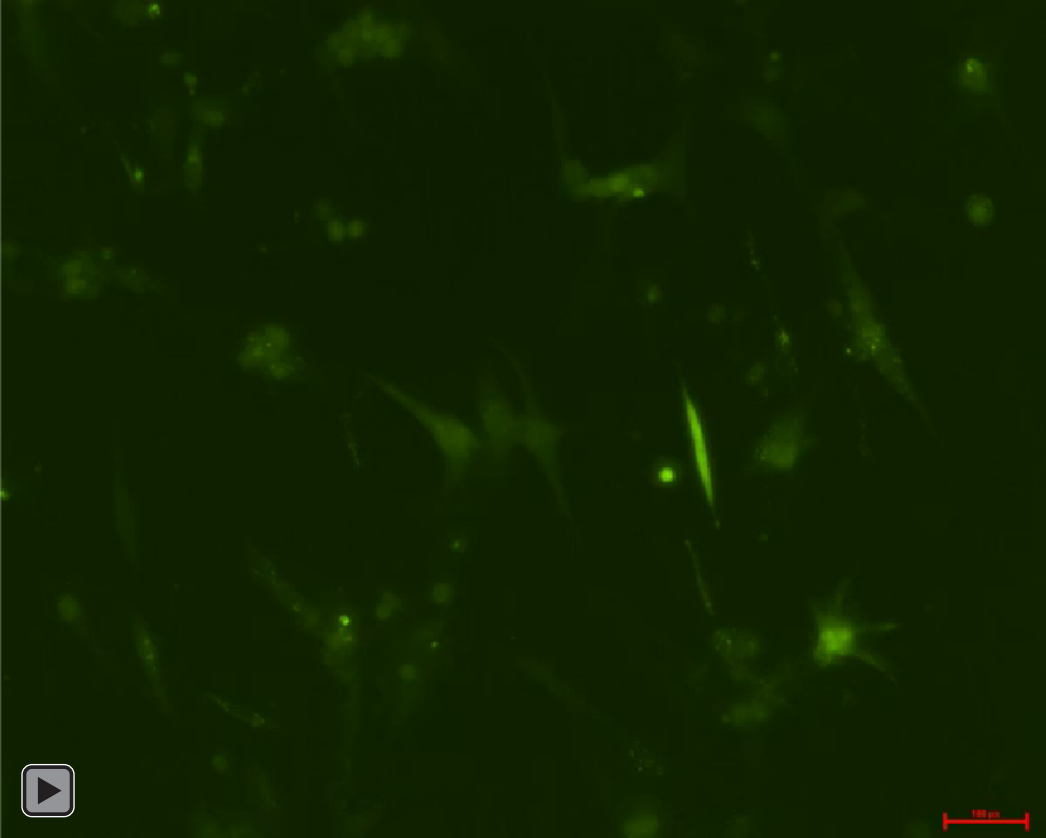
Movie S1. A real-time video showing $Ca^{2+}$ flux pattern of NRCs when grown on oxidized SACNT films, stained by Fluo-4 AM.


100 µm