

Interfacing Silicon Nanowires with Mammalian Cells

Woong Kim,[†] Jennifer K. Ng,[§] Miki E. Kunitake,[‡] Bruce R. Conklin,^{*,§} and Peidong Yang^{*,†,‡}

The Molecular Foundry and Materials Sciences Division, Lawrence Berkeley National Laboratory, and Department of Chemistry, University of California, Berkeley, California 94720, and Gladstone Institute of Cardiovascular Disease, San Francisco, California 94158

Received March 1, 2007; E-mail: p_yang@uclink.berkeley.edu; bconklin@gladstone.ucsf.edu

Nanotechnology has received increased attention in the biological research field. The important examples are (1) the usage of nanoparticles in optical and magnetic resonance imaging;^{1,2} (2) the demonstration of potential application of metal nanoshells and carbon nanotubes for the treatment of tumor and cancer cells;^{3,4} and (3) the application of nanowire-based transistors to electrically detect specific biomolecules.^{5,6} In all of these cases, the nanomaterials are functioning either inside the cells or at the vicinity of the surface of biomolecules. Direct interconnection of the cells to the external world by interfacing nanomaterials may afford great opportunities to probe and manipulate biological processes occurring inside the cells, across the membranes, and between neighboring cells.^{7,8} A nanoscale material with high aspect ratio is a good candidate for this application. For instance, silicon nanowires (SiNWs, $d = 1\text{--}100\text{ nm}$) are a few orders of magnitude smaller in diameter than mammalian cells ($d_{\text{cell}} \sim$ on the order of $10\ \mu\text{m}$) yet comparable to the sizes of various intracellular biomolecules. The nanowires have high aspect ratio ($<10^3$) and yet are sufficiently rigid to be mechanically manipulated. The nanometer scale diameter and the high aspect ratio of SiNWs make them readily accessible to the interiors of living cells, which may facilitate the study of the complex regulatory and signaling patterns at the molecular level.

In this Communication, we present the first demonstration of a direct interface of silicon nanowires with mammalian cells such as mouse embryonic stem (mES) cells and human embryonic kidney (HEK 293T) cells without any external force. The cells were cultured on a silicon (Si) substrate with a vertically aligned SiNW array on it. The penetration of the SiNW array into individual cells naturally occurred during the cell incubation. The cells survived up to several days on the nanowire substrates. The longevity of the cells was highly dependent on the diameter of SiNWs. Furthermore, successful maintenance of cardiac myocytes derived from mES cells on the wire array substrates was observed, and gene delivery using the SiNW array was demonstrated.

SiNWs were synthesized vertically aligned with respect to Si-(111) substrates via chemical vapor deposition as described earlier.⁹ The diameter of the nanowires was controlled by the size of gold nanoparticles that were used as catalytic seeds for the nanowire synthesis or by reducing the diameter of Si nanowires via oxidation and subsequent hydrofluoric (HF) acid etching step.¹⁰ The SiNW substrates had a native oxide layer and were used without any surface modification unless otherwise specified. Before any exposure to living cells, the substrates were sterilized in a solvent of 70% ethanol and 30% sterile water.

First, physical interaction between the nanowires and the cells was studied using confocal microscopy and scanning electron microscopy (SEM). Mouse embryonic stem cells stably expressing

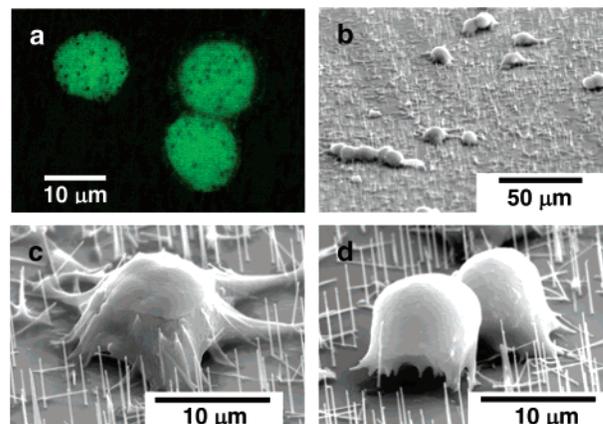


Figure 1. (a) A confocal microscopy image of mouse embryonic stem (mES) cells penetrated with silicon nanowires. (b) SEM image of mES cells on a nanowire array substrate. (c, d) SEM images of individual mES cells penetrated with silicon nanowires. The diameter and the length of the nanowires are $\sim 90\text{ nm}$ and $\sim 6\ \mu\text{m}$, respectively.

green fluorescent protein (GFP) were cultured on silicon nanowire array substrates in standard non-differentiation medium. To facilitate visualization, a nanowire array was prepared with a high density (20–30 nanowires/cell). The diameter and the length of the nanowires were $d \sim 90\text{ nm}$ and $L \sim 6\ \mu\text{m}$, respectively. 50 000–100 000 cells were incubated with a substrate deposited in a well of a 24-well plate. As the cells in the culture medium subsided on the nanowire substrate, the cells were penetrated by silicon nanowires as revealed by microscopy. Figure 1a shows a confocal microscopy image of the pierced cells. The nanowires appeared as black dots inside the cells. The focal plane was adjusted to the top of the nanowires inside the cells. Each dot corresponds to a local area of a cell that was occupied by each nanowire. SEM images of the samples are shown in Figure 1b–d. To maintain the morphology of the cells for SEM, the samples were prepared via a critical point drying technique after the treatment with glutaraldehyde for fixation and osmium tetroxide for contrast enhancement. Figure 1c,d shows individual cells penetrated by silicon nanowires. Several nanowires inside and underneath the cells can be clearly seen. The penetration was routinely observed within an hour of the incubation by fluorescent microscopy. No external force was necessary for the penetration, owing to the small diameter and high aspect ratio of nanowires. The results indicate that nanowires can be readily introduced inside the cells.

Next, viability of the penetrated cells was studied via the proliferation of the cells and propidium iodide (PI) staining. To ensure that we study only the cells that were pierced with nanowires, the substrates were first placed in a culture medium containing cells for an hour for the cell subsidence and then transferred into fresh medium without cells for further incubation. The density of

[†] Lawrence Berkeley National Laboratory, University of California, Berkeley.

[‡] Department of Chemistry, University of California, Berkeley.

[§] Gladstone Institute of Cardiovascular Disease.

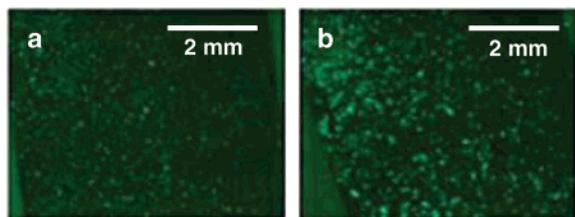


Figure 2. Proliferation of mES cells cultured on a silicon nanowire array. Fluorescent microscopy images taken (a) after 1 day, and (b) after 3 days.

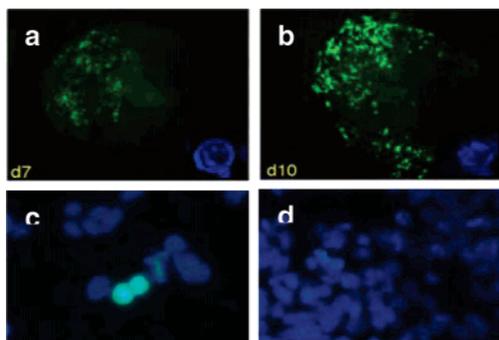


Figure 3. Differentiation of embryoid bodies of mES cells observed (a) on day 7 of differentiation (inset: nucleus of each cell stained with DAPI), and (b) on day 10 of differentiation. Human embryonic kidney cell line (HEK 293T) cultured on SiNWs (c) with and (d) without PEI treatment prior to GFP plasmid deposition.

nanowires was adjusted so that each cell could be pierced by 2–3 nanowires on average. The length of the nanowires was 3–6 μm . Three different diameters of SiNW were used: $d \sim 30, 90,$ and 400 nm. Figure 2a,b shows the green fluorescence of mES cells on 90 nm SiNWs observed after 24 and 72 h of incubation, respectively. The cells survived and proliferated for up to 3 days. The loss of cell viability was quantitatively measured with PI staining after 48 h of the incubation; 22% of the total adhered cells were stained with PI, while 78% of the cells remained healthy. The cell viability showed strong dependence on the size of the wires. When the larger diameter ($d \sim 400$ nm) of the SiNW was used, cell death occurred within a day, whereas cells grown on smaller diameter ($d \sim 30$ nm) SiNWs were alive more than 5 days. The biocompatibility of small diameter nanowires is a critical element for in situ study of cellular processes of living cells. How the penetration affects the functions of the cells will be studied in more detail in the future.

Then, the SiNW array substrates were evaluated as a new type of substitute for conventional gelatin coated tissue culture grade plastic plates for maintenance of differentiated stem cells. 90 nm diameter SiNWs were used with an average of 2–3 NWs/cell density for the rest of the experiments. First, embryoid bodies (EBs) composed of mES cells expressing GFP under the control of the β -myosin heavy chain promoter were formed via the hanging drop method.^{11–13} At day 7 of differentiation, the EBs expressing GFP, and thus containing cardiac myocytes, were transferred onto the silicon nanowire substrate. Figure 3a,b shows the proliferation and the further differentiation of the mES cells on a nanowire substrate, as indicated by increasing number of GFP expressing cells from day 7 to day 10. The beating motion of EBs became clear over time, as well. The EBs continued to grow and beat, which was monitored for more than a month. It should be noted that only the bottom layer of the cells directly contact the SiNWs.

Finally, we report a preliminary result of gene delivery using a SiNW array. The HEK 293T cell line was cultured on SiNW

substrates with DNA that was electrostatically deposited. The substrate was, first, soaked in a positively charged polymer, polyethylene imine solution (PEI, 10% in water), to facilitate the deposition of oppositely charged DNA.¹⁴ After thorough rinsing with DI water and N_2 drying step, the substrate was deposited with 2 μg of plasmid DNA (0.2 $\mu\text{g}/\mu\text{L}$) construct containing GFP under the control of the cytomegalovirus promoter. 100 000–200 000 cells were then incubated on the substrates. A day later, some cells expressed GFP (<1%), indicating successful delivery and normal function of the exogenous gene (Figure 3c). Figure 3d shows the HEK cells grown on the SiNW substrate treated with no PEI. The nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI). Interestingly, no HEK cells adhered to flat silicon substrates under the same culturing conditions. This indicates that the penetration of nanowires promotes the retention of the cells on the substrates and therefore the gene delivery. We hypothesize that the low efficiency of transfection might be due to the difficulty of releasing the electrostatically bound DNA and can be improved with more sophisticated conjugation and release scheme, for example, using disulfide bond linkage.

In summary, we demonstrated that mammalian cells can be cultured on vertically aligned SiNW array substrates. In spite of the physical penetration of nanowires, the cells survived for up to a week. Observations of continued differentiation of mES cells on SiNW array substrates were comparable to those seen with cells grown on gelatin coated tissue grade plastic. Gene delivery using a SiNW array was demonstrated. Our results suggest that the nanowires can be potentially utilized as a powerful tool for studying intra- and intercellular biological processes.

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Supporting Information Available: Quantitative data of the propidium iodide staining and the cell adhesion experiment. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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