Research Focus

Penetrating living cells using semiconductor nanowires

Stephen J. Pearton[1], Tanmay Lele[2], Yiider Tseng[2] and F. Ren[2]

[1] Department of Materials Science and Engineering, University of Florida, Gainesville, FL32611, USA
[2] Department of Chemical Engineering, University of Florida, Gainesville, FL 32611, USA

A recent publication by Kim *et al.* on penetrating both human embryonic kidney cells and mouse embryonic stem cells with Si nanowires highlights the increasing interest in using proven semiconductor materials not only to detect specific biomolecules in solutions but also to deliver genetic material or potentially screen for the presence of particular molecules at the cell level. Many semiconductors are biocompatible and this recent work has shown that penetrating cells with large diameters compared with those of the semiconductor nanowire is not fatal to the cell and that the cells remain functional for a few days.

That's got to hurt!

Everyone is familiar with the success of modern microelectronic devices that have brought us the information age, starting with the invention of the Si transistor 60 years ago. The success of these devices relies on the growth of thin films of the particular semiconductor. Much less utility has been made of so-called one-dimensional semiconductor structures called nanowires or nanorods. One can make small transistors and lasers and so on readily with these structures, however, a drawback occurs in how to interface them together in the kinds of numbers used in conventional integrated circuits, in which tens of millions of devices are harnessed together to provide the data-processing or storage functionality. There has been published recently an intriguing report of culturing mammalian cells on Si nanowire arrays, which penetrate the living cells (but do not kill them if the diameter of the nanowires is small compared with the diameter of the cells) [1]. Note that the cell is not anchored by the nanowires but attaches to the underlying Si substrate as it settles and engulfs the nanowires. The vast majority of mammalian cells inside the body require adhesion to a solid substratum for survival. Exciting new strategies have emerged recently to control mammalian cell behavior by engineering the nanoscale properties of the cell substratum. In the report from Kim *et al.* [1], the Si nanowires were first prepared on a standard Si substrate with a density such that there were 20–30 nanowires within the diameter of a typical cell. The vertically aligned Si nanowires were approximately 6 μm long with a diameter of 90 nm. Mouse embryonic stem cells were cultured on these substrates and were penetrated by the Si nanowires within approximately 1 hour as they settled on to the substrate without the application of external force. The cells survived this process provided the Si nanowire diameter was small compared with the cell size. Figure 1 shows both a confocal microscope image (Figure 1a) and scanning-electron microscope image (Figure 1b) of the mouse embryonic stem cells penetrated by the Si nanowires. By first depositing DNA onto the wires, the researchers were also able to transfer the genetic material into human embryonic kidney cells. The team members expect that the delivery efficiency could be improved by adjusting the surface chemistry of the nanowires. The nanowire arrays could be used for drug-delivery applications or for electrical stimulation and detection in cells. Perhaps the most interesting finding of this study is the fact that cells on sparse Si nanowires get impaled by the nanowires and yet remain intact over several days of culture, even enabling the differentiation of mesenchymal stem cells into cardiac myocytes. When the nanowires were functionalized with DNA, the DNA could be delivered into the cell successfully. Interestingly, cell-survival increased as the diameter of the nanorod was reduced from 400 to 30 nm. These findings provide a novel way of introducing foreign material into the cell by engineering the cell substratum. Although such methods are complementary to other techniques that involve delivery through nanoparticles [2], the advantage is that the delivery technique is integrated with the cell substratum.

What else can nanostructures contribute?

Fundamental cell behaviors, such as motility, adhesion, proliferation and differentiation, can be exquisitely sensitive to nanoscale adhesive ligand clustering [3,4] and nanoscale topography [5–7]. Such approaches find potential applications in the design of orthopedic implants or vascular stents [8,9]. There has been great interest in the use of nanostructured materials for both tissue engineering and drug delivery [10]. This recent study has shown a new potential application for nanotopography of the cell substratum: delivery of reagents into the cell [1].

Recent work has also appeared using a related technique called nanotube spearing, which drives drugs into the cell. The transfection efficiencies are relatively high [11]. However, the disadvantage is that they need an oscillating magnetic field followed by a static field to first spear and then drive the nanotubes inside. The nanotubes are not integrated with the substrate itself, as is the case with the Si nanowire approach. One exciting area for

Corresponding author: Pearton, S.J. (spear@mse.ufl.edu).

 0167-7799/$ – see front matter doi:10.1016/j.tibtech.2007.07.011

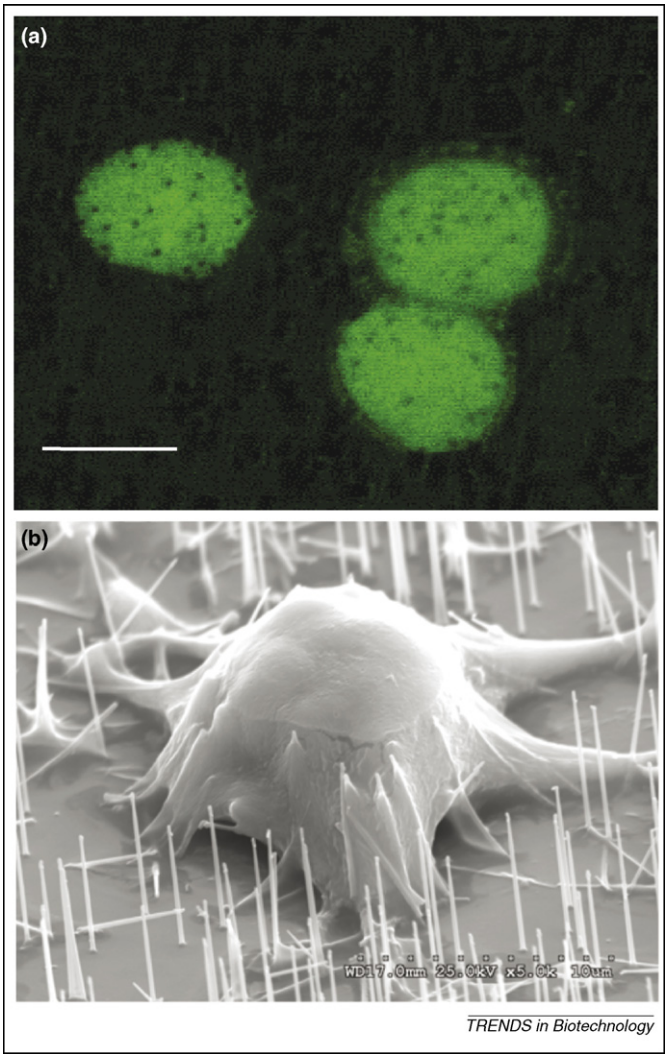

Figure 1. **(a)** Confocal microscope image and **(b)** SEM image of mouse embryonic stem cells penetrated by Si nanowires on a Si substrate. The dark spots within the cells are nanowires and the cells are stably expressing GFP. Reprinted from Kim *et al.* [1] and with permission from the American Chemical Society. The scale bar represents 10 μm.

future study is the potential use of nanowires as intracellular sensors. Carbon nanotubes have already been used as intracellular glucose sensors [12]. Similarly, if intracellular nanowire sensors integrated with substrate are developed and functionalized to detect intracellular signaling, they can be used for developing novel high throughput platforms for small-molecule drug screening. Important advances can be expected when the probing wires become functional electrically [13]. There is clearly increasing interest in the use of nanowires as penetrating agents and this is gaining acceptance. The concepts surrounding this approach have been developed in recent years by several other groups [14–17].

Important questions remain: first, the mechanism underlying cellular engulfment of the nanorods remains to be explored. The engulfment could potentially use the endocytic pathway under some conditions. Second, the range of molecules (proteins, RNA, plasmid DNA) that can be delivered with this technique needs to be established. Third, it is unclear if the source of DNA that entered the cell is from the nanowires or the substrate itself. Either case is potentially feasible given the relatively low (<1%) transfection efficiencies observed. But this paper has succeeded in a new approach to introducing foreign molecules inside cells and is generating considerable excitement.

References

1 Kim, W. *et al.* (2007) Interfacing silicon nanowires with mammalian cells. *J. Am. Chem. Soc.* 129, 7228–7229
2 Farokhzad, O.C. *et al.* (2006) Targeted nanoparticle–aptamer bioconjugates for cancer chemotherapy *in vivo*. *Proc. Natl. Acad. Sci. U. S. A.* 103, 6315–6320
3 Kong, H.J. *et al.* (2005) FRET measurements of cell-traction forces and nano-scale clustering of adhesion ligands varied by substrate stiffness. *Proc. Natl. Acad. Sci. U. S. A.* 102, 4300–4305
4 Maheshwari, G. *et al.* (2000) Cell adhesion and motility depend on nanoscale RGD clustering. *J. Cell Sci.* 113, 1677–1686
5 Zhu, X. *et al.* (2005) Fabrication of reconfigurable protein matrices by cracking. *Nat. Mater.* 4, 403–406
6 Teixeira, A.I. *et al.* (2006) The effect of environmental factors on the response of human corneal epithelial cells to nanoscale substrate topography. *Biomaterials* 27, 3945–3954
7 Popat, K.C. *et al.* (2007) Osteogenic differentiation of marrow stromal cells cultured on nanoporous alumina surfaces. *J. Biomed. Mater. Res. A* 80, 955–964
8 Sato, M. and Webster, T.J. (2004) Nanobiotechnology: implications for the future of nanotechnology in orthopedic applications. *Expert Rev. Med. Devices* 1, 105–114
9 Choudhary, S. *et al.* (2007) Enhanced functions of vascular cells on nanostructured Ti for improved stent applications. *Tissue Eng.* 54, 134–138
10 Goldberg, M. *et al.* (2007) Nanostructured materials for applications in drug delivery and tissue engineering. *J. Biomater. Sci. Polymer Edn* 18, 241–268
11 Cai, D. *et al.* (2005) Highly efficient molecular delivery into mammalian cells using carbon nanotube spearing. *Nat. Methods* 2, 449–454
12 Barone, P.W. *et al.* (2005) *In vivo* fluorescence detection of glucose using a single-walled carbon nanotube optical sensor: design, fluoro-phore properties, advantages, and disadvantages. *Anal. Chem.* 77, 7556–7562
13 Stern, E. *et al.* (2007) Label-free immunodetection with CMOS-compatible semiconducting nanowires. *Nature* 445, 519–522
14 Hultgren, A. *et al.* (2003) Cell manipulation using magnetic nanowires. *J. Appl. Phys.* 93, 7554–7556
15 Prina-Mello, A. *et al.* (2006) Internalization of ferromagnetic nanowires by different living cells. *J. Nanobiotechnology* 4, 9–14
16 Patolsky, F. *et al.* (2006) Detection, stimulation, and inhibition of neuronal signals with high-density nanowire transistor arrays. *Science* 313, 1100–1104
17 Bindal, A. and Hamedi-Hagh, S. (2007) An integrate and fire spiking neuron using silicon nano-wire technology. In *Technical Proceedings of the 2007 NSTI Nanotechnology Conference and Trade Show* (Vol. 1), pp. 173-176, Nano Science and Technology Institute