BIONANOELECTRONICS

Getting close to the action

Two independent groups have demonstrated that nanoscale electrodes can record action potentials in neurons and cardiac muscle cells, and a third group has shown that nanowire field-effect transistors can make electrical measurements on biological materials with unprecedented spatial resolution.

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Ever since Luigi Galvani and Alessandro Volta initiated a debate on the bioelectricity of nerves and muscles in the late 1800s, scientists have been trying to improve our understanding of this phenomenon and to develop tools and devices that can interface with those cells that exhibit bioelectricity. These tools have progressed from crudely wired Leyden jars to sophisticated amplifiers with miniature electrodes. Now, writing in *Nature Nanotechnology*, three independent groups — led by Charles Lieber of Harvard University[1], Hongkun Park, also of Harvard[2], and Bianxiao Cui and Yi Ciu of Stanford University[3] — report that they have down-sized electrodes to the nanoscale, and used them to record action potentials inside a variety of cells. Moreover, as these electrodes are fabricated on planar strata, they are well suited for cell-culture applications.

Park and co-workers build on previous work in which they used a vertical nanowire electrode array (VNEA) to deliver biomolecules into living cells, including neurons[4]. In the present work[2] they use standard silicon nanofabrication processes to make VNEAs that contain 16 stimulation/recording pads, with each pad containing a 3 × 3 array of nanowires. Moreover, each stimulation/recording pad can be addressed individually. The nanowires are made of highly doped silicon, which can conduct electricity almost as well as a metal, and they are encapsulated by an insulating glass (silicon dioxide) shell, and capped by a conducting metal tip (made of titanium and gold), which is the only part of the array to make electrical contact with the sample. The nanowires are 150 nm in diameter, 3 μm in height, and are spaced 2 μm apart. When neurons from the cortex of a rat were cultured on top of the VNEAs and visualized using confocal microscopy, some nanowires were engulfed by neurons, whereas others seemed to penetrate through the membrane of the cell (Fig. 1a).

Park and co-workers performed extensive characterization of the VNEA, which involved comparing its electrical performance with that of the well-established patch-clamp technique on a model cell system, and then they checked if the nanowires were able to penetrate the membranes of nerve cells. They found that some nanowires spontaneously penetrated the membrane, whereas other nanowires only entered the cells in the presence of applied electric fields (a process called electroporation). They also showed that it was possible to evoke action potentials by injecting current through the nanowire (the action potential was recorded by a patch pipette). Vice versa, the nanowires could also record action potentials evoked by current injections through the patch pipette.

The amplitude of the action potentials recorded by the nanowires was dampened ~10-fold compared with the amplitudes recorded by a patch pipette. This is expected because the nanowires have larger axial resistances than patch pipettes (~300 MΩ compared with 2–10 MΩ), whereas the seal resistance between nanowires and the cell membrane is smaller than that between the pipette and the membrane (0.1–0.5 GΩ compared with more than 2 GΩ). When single action potentials were recorded, the signal-to-noise ratio for the VNEA approach was ~100, but it was possible to improve on this by a factor of 10 by averaging repeated trials. Although this is less than has been achieved with patch clamping, Park and co-workers were still able to demonstrate the multiplex stimulation capability of the VNEA platform in experiments on neuronal networks that contained multiple presynaptic neurons connected, by chemical synapses, to a single postsynaptic neuron of interest: different VNEA pads were used to evoke action potentials in the presynaptic neurons, and patch clamping was used to record the responses at the postsynaptic neuron.

This demonstration of neuronal network mapping represents a real advantage of the VNEA device over the standard electrophysiological approaches. Using patch-clamp recording, for example, to determine the synaptic connectivity would be a laborious process: one would keep the postsynaptic neuron in the whole-cell mode, while moving a second glass microelectrode from neighbouring neuron to neuron to evoke their action potentials[5]. However, a number of alternative approaches — notably, scanning laser photostimulation of a caged neurotransmitter[6] and optogenetic stimulation using genetically encoded light-sensitive channels[7] — can also be used to map synaptic connectivity.

The Stanford researchers also build on previous work. In 2010 they used arrays of vertical nanopillar electrodes on individually addressable platinum pads to study neuronal growth and mobility[8]. Now they have used the same platform to make both extracellular and intracellular recordings of the action potentials in cardiac muscle cells (cardiomyocytes) derived from mice[3]. The nanopillars, which were also made of platinum, were 150 nm in diameter and 1–2 μm in height, and unlike the nanowires used by the Park group, all of the nanoelectrode made electrical contact with the sample in the Stanford experiments. Cui and co-workers[3] varied the number of pads and the number of nanoelectrodes per pad, and found that, as expected, the impedance (that is, the electrical resistance to an alternating current) measured in the cell culture medium decreased as the number of nanoelectrodes per pad increased from 3 to 5 to 9.

Cells cultured on top of the arrays displayed spontaneous contractions driven by action potentials, and the Stanford group recorded these action potentials with nanopillar electrodes in both the extracellular mode and (after electroporation) the intracellular mode. The shape and duration of recorded action potentials were similar to those previously recorded with the patch-clamp approach, but the amplitudes measured with the nanopillar electrodes were lower by a factor of about 10 (and also decayed over time), and the signal-to-noise ratio was also fairly low (~7). However, Cui and co-workers were able to demonstrate the multiplexing

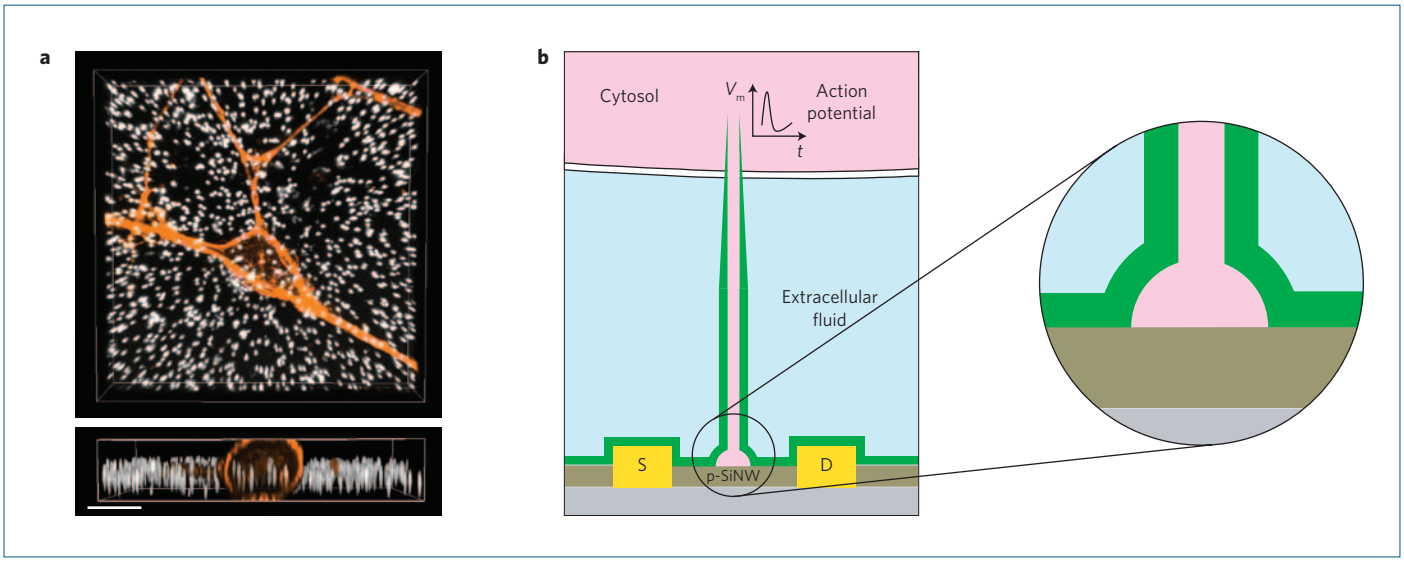

Figure 1 | Getting inside cells. **a**, Park and co-workers used VNEAs to record action potentials in neurons[2]. This three-dimensional reconstruction (shown as a top view and a side view), which is based on data acquired with laser confocal scanning microscopy, shows that the silicon nanowires (white) entered the cells (orange). Scale bar is 10 μm. **b**, In the BIT-FET developed by Lieber and co-workers[1], a hollow nanotube made of silicon dioxide (green) penetrates the cell membrane (white), bringing the cytosol (pink) into contact with a doped silicon nanowire (p-SiNW). Changes in the voltage of the cell membrane (V_m) over time (inset) influence the current flowing from the source electrode (S) to the drain electrode (D). This schematic is not to scale.

capability of their approach by making simultaneous recordings from several cells over four days. They also demonstrated that devices based on nanopillar electrodes could have applications in drug screening by detecting small changes in the action potentials induced by drugs that target ion channels in cells.

Both the Park and the Cui groups essentially developed nanoscale versions of the conventional microelectrode array. Although this reduced the invasiveness of the approach for intracellular recordings, there was no notable increase in spatial resolution because this measure is determined by the size of the array on a pad, not the diameter of the individual electrodes. Moreover, all electrodes suffer from increases in impedance as they become smaller. One way to lower the impedance of metal microelectrodes is to coat them with carbon nanotubes[9] or use vertically aligned carbon nanofibres[10] and exploit the fact that these materials can be ballistic conductors (that is, they do not notably reduce the speed of electrons moving through them). There have been proposals to use arrays of individually addressable vertically aligned single-walled carbon nanotubes to record synaptic activity[11] but such devices are beyond what is technologically possible at present. There is, therefore, a pressing need for approaches that minimize the interface between the probe and the cell, and hence minimize the impact of the measurement on the cell, without unduly increasing the impedance.

Lieber and co-workers report an innovative way to avoid the problems associated with impedance[1]. Previously they have used devices based on the field-effect transistor (FET) to make extracellular recordings of neurons and cardiomyocytes[12,13], and as the performance of a FET does not depend on impedance, FET-based devices are an excellent choice for making intracellular measurements as well. For their latest work Lieber and co-workers have developed a new type of device called a branched intracellular nanotube FET (BIT-FET; Fig. 1b). To make these devices they started by depositing doped silicon nanowires with diameters of 100 nm on a silicon nitride substrate, and then grew germanium nanowires that branched from the doped silicon ones at nearly right angles. Next they added titanium/palladium source and drain contacts to the doped silicon nanowire, on either side of the germanium branch, to make a nanowire FET, and then covered everything with silicon dioxide. Finally they removed the silicon dioxide at the tip of the branch and used hydrogen peroxide to remove the germanium, which left a hollow nanotube made of silicon dioxide protruding from the doped silicon nanowire FET. This nanotube was 1–1.5 μm in height, with an outer diameter that tapered from ~150 nm at its base to ~55 nm at its tip. The tube had an inner diameter of 50 nm, except at its wider base, and an internal volume of ~3 attolitres (Fig. 1b).

Once the open end of the nanotube has entered a cell, the intracellular liquid (known as the cytosol) comes into contact with the channel of the FET (which runs from the source contact to the drain contact). Because the intracellular liquid is conducting, changes in the voltage of the cell membrane influence the gate voltage of the FET, leading in turn to changes in the current through the channel. Therefore, by measuring the current through the FET over time it is possible to record action potentials in the cells (Fig. 1b).

Lieber and co-workers started by characterizing the electrical properties of the BIT-FET using phosphate-buffered saline and determined that the time resolution was about 0.1 ms. Then they cultured cardiomyocytes from chickens on a sheet of an elastomeric mould[13], which they lowered onto a BIT-FET that had been coated with a phospholipid bilayer to help the nanotube penetrate the cell membrane[14]. At first the recordings were clearly extracellular, but they became intracellular less than a minute after the mould containing the cardiomyocytes had been placed on the device. This was evident from the recorded action potentials being coincident with the beating of the cell. Moreover, the shape and amplitude (~75–100 mV) of the action potentials were similar to those recorded previously with the patch-clamp technique. The signal-to-noise ratios were 40–80 (C. M. Lieber, personal communication). Furthermore, when the recording was stopped — either deliberately, or because contraction had caused the cell to move away from the BIT-FET — it was possible

to re-establish contact and start recording again. Indeed, Lieber and co-workers managed to do this up to five times over a recording period exceeding one hour.

The Harvard team also made simultaneous recordings from multiple BIT-FETs either from a single cardiomyocyte or from a network of these cells. And based on computational modelling, they predict that it should be possible to make intracellular recordings of action potentials using BIT-FETs in which the inner diameter of the nanotube is as small as 3 nm. It should be noted that reducing the diameter of the nanotube will not reduce the sensitivity of the device because the signal-to-noise ratio is largely determined by the area over which the channel of the FET and the cytosol are in contact with each other. It should be possible to adjust the nanofabrication process used to build the BIT-FETs to make this area as large as possible (to maximize the signal-to-noise ratio), while reducing the diameter of the nanotubes (to improve the spatial resolution).

These three papers[1–3] represent substantial progress in the development of experimental techniques for electrophysiology that are minimally invasive and have the potential to be used in multiplexed measurements. As the three platforms can be readily interfaced with optical imaging, it should be possible to combine them with photostimulation methods. Furthermore, owing to their small size and potential for high-density packing, they could represent a new generation of probes for brain/muscle–machine interfacing. Although the three techniques have lower signal-to-noise ratios than conventional glass micropipettes, there is scope for improvement.

The BIT-FET also offers unprecedented spatial resolution for an electrical recording technique that can be used on biological materials. For instance, BIT-FETs should be able to make electrical recordings of dendrites — branched structures that project from neurons (and have diameters that are much smaller than the body of the neuron). Although patch pipettes have been used to make recordings from neuronal dendrites[15], these measurements are laborious and fairly invasive. The small size of the nanotube in a BIT-FET makes this approach well suited to making measurements on dendrites and, possibly, on individual dendritic spines (which are the small 'branches' found in some dendrites).

The next challenge for all these approaches will be to develop techniques to position the probes over and within subcellular components of interest, and to devise ways of using the same probes to both stimulate and record action potentials for subcellular measurements. Nonetheless, the nanoscale probes developed by the two Harvard groups and the Stanford group hold great promise for the investigation of bioelectricity in nerve cells and cardiac muscle, and for studying intercellular connectivity to learn more about health and disease. ❐

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