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Optimizing Nanoelectrode Arrays for Scalable Intracellular Electrophysiology

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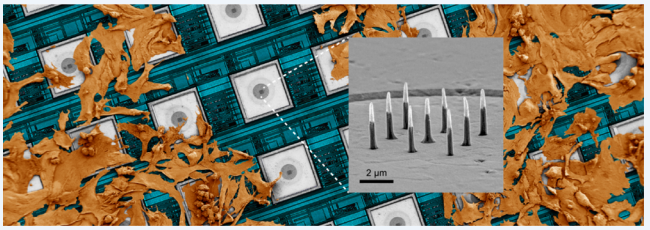
CONSPECTUS: Electrode technology for electrophysiology has a long history of innovation, with some decisive steps including the development of the voltage-clamp measurement technique by Hodgkin and Huxley in the 1940s and the invention of the patch clamp electrode by Neher and Sakmann in the 1970s. The high-precision intracellular recording enabled by the patch clamp electrode has since been a gold standard in studying the fundamental cellular processes underlying the electrical activities of neurons and other excitable cells. One logical next step would then be to parallelize these intracellular electrodes, since simultaneous intracellular recording from a large number of cells will benefit the study of complex neuronal networks and will increase the throughput of electrophysiological screening from basic neurobiology laboratories to the pharmaceutical industry. Patch clamp electrodes, however, are not built for parallelization; as for now, only ∼10 patch measurements in parallel are possible.



It has long been envisioned that nanoscale electrodes may help meet this challenge. First, nanoscale electrodes were shown to enable intracellular access. Second, because their size scale is within the normal reach of the standard top-down fabrication, the nanoelectrodes can be scaled into a large array for parallelization. Third, such a nanoelectrode array can be monolithically integrated with complementary metal-oxide semiconductor (CMOS) electronics to facilitate the large array operation and the recording of the signals from a massive number of cells. These are some of the central ideas that have motivated the research activity into nanoelectrode electrophysiology, and these past years have seen fruitful developments. This Account aims to synthesize these findings so as to provide a useful reference.

Summing up from the recent studies, we will first elucidate the morphology and associated electrical properties of the interface between a nanoelectrode and a cellular membrane, clarifying how the nanoelectrode attains intracellular access. This understanding will be translated into a circuit model for the nanobio interface, which we will then use to lay out the strategies for improving the interface. The intracellular interface of the nanoelectrode is currently inferior to that of the patch clamp electrode; reaching this benchmark will be an exciting challenge that involves optimization of electrode geometries, materials, chemical modifications, electroporation protocols, and recording/stimulation electronics, as we describe in the Account. Another important theme of this Account, beyond the optimization of the individual nanoelectrode−cell interface, is the scalability of the nanoscale electrodes. We will discuss this theme using a recent development from our groups as an example, where an array of ca. 1000 nanoelectrode pixels fabricated on a CMOS integrated circuit chip performs parallel intracellular recording from a few hundreds of cardiomyocytes, which marks a new milestone in electrophysiology.

1. INTRODUCTION

Electrode-based measurements of the membrane potentials of electroactive cells have enabled fundamental discoveries in neuroscience and cardiology. While extracellular electrodes (Figure 1b) detect action potentials, intracellular electrodes (Figure 1a, c) have a greater sensitivity and can measure the full spectrum of membrane potentials, including not only the action potentials but also the much smaller postsynaptic potentials. Traditionally, the patch clamp technique (Figure 1a) has been the dominant electrode-based method for intracellular electrophysiology: an ultrasharp glass pipet, filled with ionic solution and an electrode, patches a cell membrane and gains intracellular access to the cytosol, permitting detailed electrical interrogation of the cell. However, the bulky mechanical setup required for precise positioning of the pipet and the elaborate patching process on individual cells prevents large-scale parallel operation.[1] In addition, continuous solution exchange between the pipet and cytosol eventually causes cell malfunction, limiting the measurement time.

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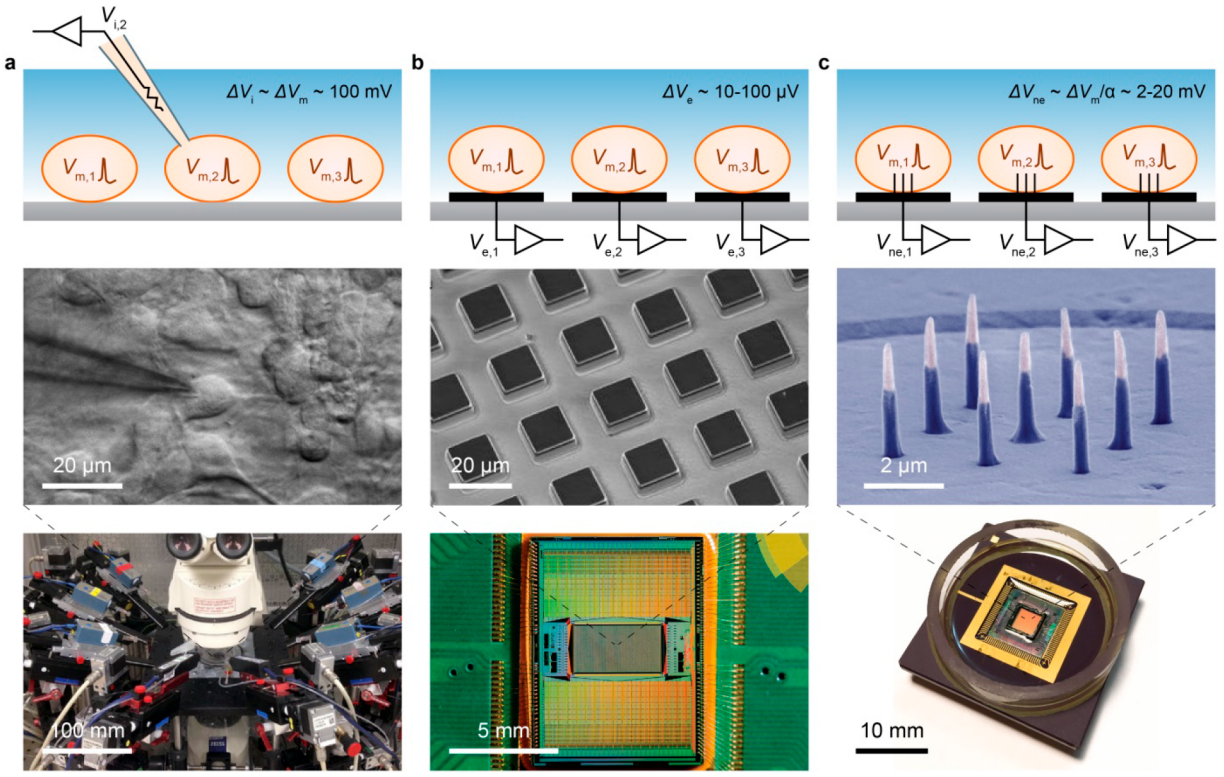

Figure 1. Intracellular and extracellular electrodes. (a) The patch clamp provides a high-fidelity intracellular interface by using a patch pipet, off-substrate electronics, and manipulators. Advanced systems can operate up to ∼10 pipettes in parallel.[1] Bottom, adapted with permission from ref 1. Copyright 2015 Springer Nature. (b) CMOS microelectrode arrays (MEAs) integrate electrodes (middle[38]) and electronics (bottom[25]) onto the same substrate, allowing for dense arrays (e.g., ∼ 65,000 electrodes).[22,25,37,38] By measuring extracellular electrophysiological signals, they are able to perform network level studies. Middle, adapted with permission from ref 38. Copyright 2017 Springer Nature. Bottom, adapted with permission from ref 25. Copyright 2015 Royal Society of Chemistry. (c) Nanoscale intracellular electrodes can be used in conjunction with CMOS technologies to combine the benefits of high-fidelity intracellular signals with network level capabilities. The example shown has ∼1000 intracellular electrodes.[2] Adapted with permission from ref 2. Copyright 2017 Springer Nature.

The drive to overcome these drawbacks of the patch clamp electrode has spurred research into nanoscale electrodes, ranging from 10 nm to ∼1 μm in size (Figure 1c). Because these electrodes can intracellularly interface with cytosol yet are smaller than typical patch clamp pipettes, solution exchange is reduced and the interrogation time can potentially be prolonged. Furthermore, as their dimensions are well within the reach of complementary metal-oxide-semiconductor (CMOS) fabrication technology, these nanoelectrodes can be produced in a highly parallel manner into a large-scale array and can be seamlessly integrated with CMOS electronic circuits (Figure 1c).[2] Such a platform will open up the possibility for high-fidelity intracellular recording of a massive number of neurons and other excitable cells. Measuring the intracellular membrane potential of even a few cells revealed important insight into neuronal network function;[3] massively parallel nanoscale electrodes will be well suited for such fundamental neuroscience inquiries as functional connectome mapping, plasticity modulation, and single-cell precision prosthetic control. In addition, the nanoelectrode array may open new avenues in drug screening, with the ability to examine pharmaceutical effects on the network behavior with high precision.[4]

In this Account, we will review recent advances on nanoelectrodes for electrophysiology. In particular, we will focus on the morphology and electrical circuit model of the nanoelectrode-cell interface and how these properties drive the optimization of individual nanoelectrodes (section 2), and the effort to scale up such nanoelectrodes into a massive scale array (section 3).

2. NANOELECTRODE–CELL INTERFACE

When a cellular membrane is placed on a nanoscale electrode protruding from the surface, the membrane can deform and wrap around it, thus excluding it from the interior of the cell (Figure 2). Many works have observed this membrane wrapping. Scanning electron microscopy (SEM)[5] of fixed cells residing on nanopillars shows membrane deformation due to vertical pillars (Figure 2a). Confocal microscopy studies, labeling the cell membrane and nanostructures separately, shows gradual settling of live cells around vertical pillars[6] with the exclusion of the pillars from the cell interior[7] (Figure 2b). Cross-sectional SEM[8] and transmission electron microscope (TEM)[9] images using focused ion beam (FIB) sectioning also confirm that membranes wrap around vertical nanostructures (Figure 2c, d).

For the nanoelectrode to gain intracellular access, it needs to bypass the cellular membrane and come into direct contact with the cytosol. Spontaneous or induced perforations in the region of the membrane that wraps around the nanoelectrode can provide such direct contact. Spontaneous perforations have been observed in the context of intracellular delivery: biomolecules adhered to vertical nanopillars were successfully delivered across the membrane into the cells.[5,6,10] Spontaneous

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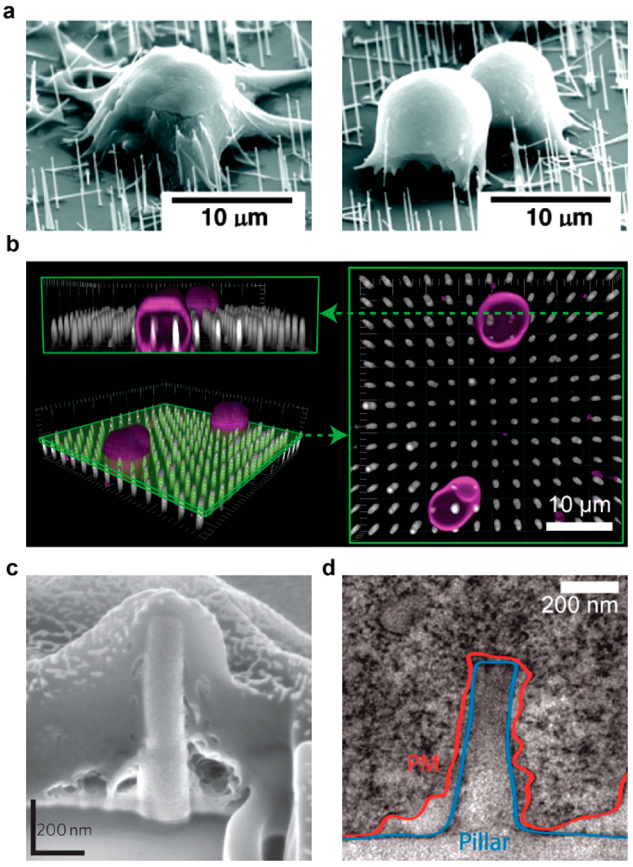

Figure 2. Characterization of the nanostructure-cell interface. (a) SEM images of cells on vertical nanowires showing the membrane deforming around the nanowires.[5] Reproduced with permission from ref 5. Copyright 2007 American Chemical Society. (b) Three-dimensional reconstruction of confocally imaged human B cells (membrane: magenta) on top of Alexa-label nanowires (white).[7] Adapted with permission from ref 7. Copyright 2012 American Chemical Society. (c) FIB-SEM cross section of a cell wrapping around a vertical nanowire.[8] Reproduced with permission from ref 8. Copyright 2012 Springer Nature. (d) TEM cross section of a cell-nanowire interfaces showing the cell's plasma membrane (PM, red) wrapping around the vertical nanowire/nanopillar (blue).[9] Reproduced with permission from ref 9. Copyright 2012 American Chemical Society.

perforations, however, may be a rare event, may be transient, and/or might not always happen at the tip of nanopillars; one study using hollow nanostraws,[11] for instance, has shown that the probability of spontaneous formation of holes at the top of the nanostraws may be as low as 7%. By contrast, induced perforations, e.g., via electroporation, are a far more controlled way to ensure direct intracellular access of the electrode into the cytosol.

2.1. Circuit Model and Electrode Optimization

Figure 3a shows a generalized circuit model of the nanoelectrode−cell interface.[2,12−15] The cell's membrane is divided into the top membrane facing the grounded extracellular solution and the bottom junctional membrane facing the nanoelectrodes (both membranes are modeled as voltage gated ion-channels with membrane capacitances). The tight solution gap between the cell membrane and the nanoelectrode/substrate is modeled as a seal resistance (R_s). The nanoelectrode uses an access resistance (R_a) to measure the intracellular potential (Figure 3c). For efficient intracellular recording, R_a must be small enough to bypass the parallel junctional membrane impedance (due to C_{jm} and the ion-channels). This can be achieved by electroporation[2,8,12−14,16,17] (e.g., Figure 3d), which generates small holes in the membrane by the application of a voltage, or by direct penetration through the membrane.[13,18−20] An attenuated version of the intracellular signal in the junctional solution then results from voltage division:

$$\frac{V_{sol}}{V_m} \approx \frac{R_s}{R_s + R_a} \quad (1)$$

For comparison, extracellular electrodes measure the junctional solution voltage without intracellular access ($R_a \sim \infty$, Figure 3b), thereby causing eq 1 to not apply. Small positive or negative signals can be recorded, as determined by the complex flow of extracellular ion channel currents across the seal resistance.[12,15] For either extracellular or intracellular recording, the signal in the junction solution is then further divided by the nanoelectrode impedance, the parasitic impedance, and the input impedance of the recording apparatus (eq 2 where these impedances are assumed to be capacitive, defined as C_{ne}, C_p, and C_{in}, respectively).

$$\frac{V_{ne}}{V_{sol}} \approx \frac{C_{ne}}{C_{ne} + C_p + C_{in}} \quad (2)$$

From these relations, to optimize the nanoelectrode−cell interface and capture the full spectrum of intracellular signals, the seal resistance and parasitic impedance should be increased (to prevent signal leakage) whereas the access resistance and nanoelectrode impedance should be decreased (for maximum voltage transfer). Furthermore, the output signal is solely dependent on the ratios of these electrical parameters, and thus the quality of the intracellular recording can be significantly improved with proper engineering and optimization of the electrode-cell and electrode-electronics interfaces.[21]

A wide variety of nanoelectrodes have been developed to gain intracellular access (Table 1, Figure 4). In the following sections, we address these efforts through the lens of how they improve each of the electrical components of the interface.

2.2. Nanoelectrode Geometry

The nanoelectrode geometry defines the morphology of the cell-to-electrode interface, which in turn, greatly affects the seal resistance in two aspects: the cell to electrode/substrate gap distance and the electrode circumference. In comparison to traditional planar electrodes, the smaller gap between nanoelectrodes and cells can result in a higher seal resistance.[22] For intracellular nanoelectrode works, pillar geometries have been the most widely used,[2,8,13,18,23] where TEM cross sections show the cell membrane is closer to the top/side of nanopillars than to the flat substrate.[9] Vertical tube geometries[14,20,24] have also been investigated where the denting of cell membranes into the nanometer scale holes improved seal resistance and prolonged recording time.[14] Mushroom shape electrodes, designed to mimic the morphology of the synaptic connection,[21] have also been shown to form a tight gap with cell membranes.

The circumference of the nanoelectrodes also affects the seal resistance. For planar electrodes, the seal resistance is roughly inversely proportional to the electrode's circumference, while the nanoelectrode's impedance is inversely proportional to the surface area. Consequently, there is a trade-off for electrode size between lower impedance and higher seal resistance. With vertical pillar geometry, however, the impedance of the

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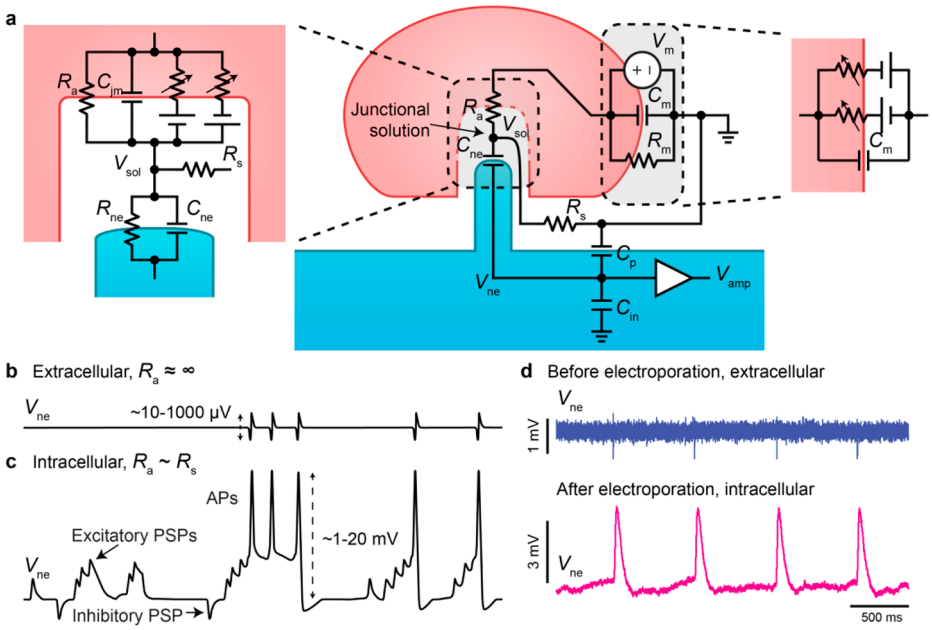

Figure 3. Circuit model for the nanoelectrode−cell interface. (a) The junctional cell membrane (left) consists of an access resistance, R_a, junctional membrane capacitance, C_{jm}, and various ion channels with their reverse potentials. The nanoelectrode-to-solution interface consists of a double layer capacitance, C_{ne}, and resistance, R_{ne}. The solution gap between the cell membrane and electrode/substrate forms the seal resistance, R_s. The model can be greatly simplified (center) by considering only the lowest impedances, R_a, C_{ne}, and R_s. The circuit model of cell membrane (right) consists of various ion channels and cell membrane capacitance, C_m, which can be simplified (center) to the membrane potential, V_m, in parallel with C_m and membrane resistance, R_m. The voltage recorded by the nanoelectrodes, V_{ne}, is an attenuated version of the solution potential, V_{sol}, in the junction between the nanoelectrode and cell; C_p and C_{in} represent the parasitic capacitance and input capacitance of the amplifier, respectively. V_{amp} is the recorded output voltage after amplification. (b, c) Extracellular and intracellular voltage signals are measured by the nanoelectrode without and with an access resistance, respectively. With intracellular access ($R_a \sim R_s$), all neuronal signals including excitatory and inhibitory post synaptic potentials (PSPs) and action potentials (APs) can be measured. Without intracellular access ($R_a \sim \infty$), an attenuated and distorted signal is measured, permitting only measurement of the action potentials. (d) Extracellular and intracellular recordings of cardiac action potentials from the same cardiomyocyte using vertical nanoelectrodes and electroporation to permeate the membrane and reduce R_a for intracellular recording.[2] Adapted with permission from ref 2. Copyright 2017 Springer Nature.

Table 1. Summary of Nanoelectrode Works for Intracellular Electrophysiology

identifier and reference	electrode geometry	cell	electrode fabrication	electrode material	access method	experiments
i (patch clamp)	glass tube	all cells		Ag/AgCl	mechanical	recording and stimulation
ii[19]	kinked nanowire	chicken cardiomyocyte	nanocluster catalyzed SiNW growth	Si n+/n/n+ FET	mechanical and lipid coating	recording
iii[23]	vertical nanowire	GH3	CVD growth and top down fabrication	Si coated with Pt	spontaneous penetration	recording
iv[13]	vertical nanowire	rat neuron	top down fabrication on SOI wafer	Si coated with Au	electroporation	recording and stimulation
iv[2]	vertical nanowire	rat cardiomyocyte	top down fabrication on CMOS IC	SiO_2 coated with Pt	electroporation	recording and stimulation
v[17,21,32]	mushroom	snail neurons and rat cardiomyocyte	electrodeposition	Au	adhesion promoting peptide[21,32] or electroporation[17]	recording
vi[24]	vertical nanotube	rat neuron, HL-1 cell (cardiac)	FIB milling	Au	optoporation	recording
vii[18]	vertical nanowire	mouse, rat, and hiPSC-derived neurons	top down fabrication on sapphire substrate	Si	spontaneous penetration	recording
viii[20]	vertical nanotube	chicken cardiomyocyte	metal catalyzed CVD growth and top down fabrication	SiO_2 wall tube	lipid coating	recording
ix[8]	vertical nanowire	HL-1 cell (cardiac)	FIB deposition	Pt	electroporation	recording
x[14]	vertical nanotube	HL-1 cell (cardiac)	electrodeposition	IrO_2	electroporation	recording

nanoelectrode can be reduced while still maintaining the seal resistance benefits of a small electrode circumference. Furthermore, multiple nanoelectrodes can be used in parallel to lower the electrode impedance.[2,8,13,14,21,24]

2.3. Electrode Material

The nanoelectrode impedance is also related to its material. The most widely used materials for nanoelectrodes are noble metals, Au[13,21,24] or Pt,[2,8] which pass current through catalyzing

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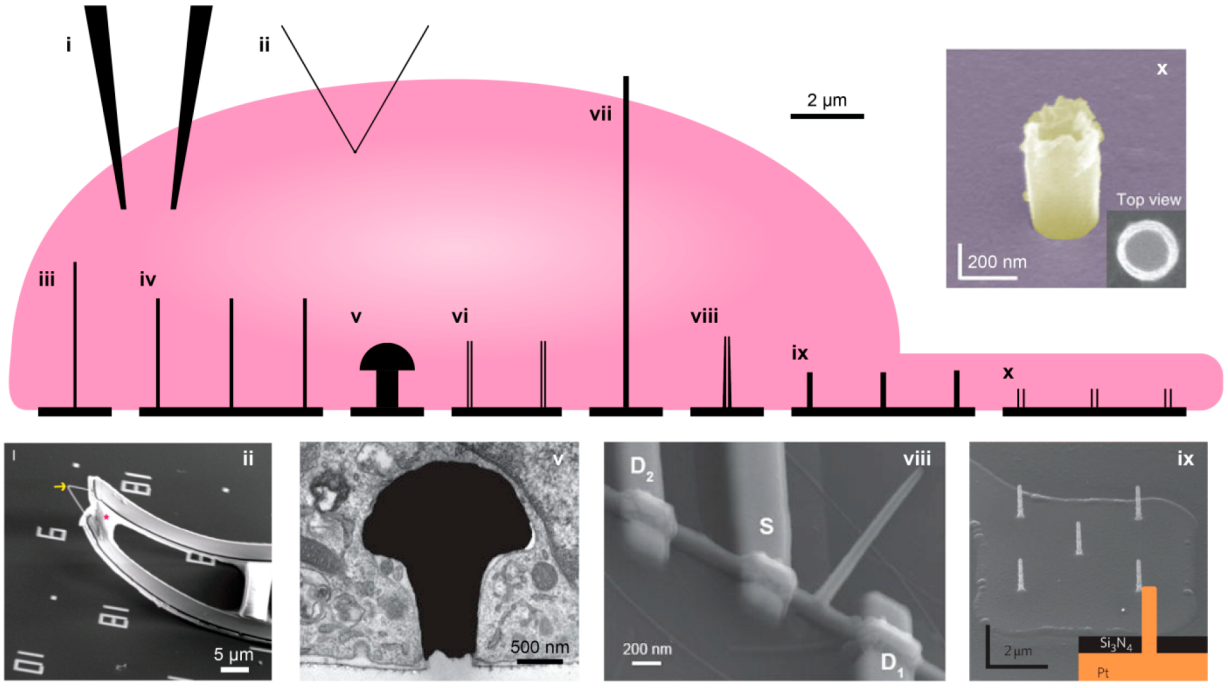

Figure 4. Various nanoelectrode geometries for intracellular recording. (i–x) Illustrations of the nanoelectrodes listed in Table 1 drawn to the same scale. The background is the typical size of a rat neuron soma and axon. Images are included from various works for example; figure concept adapted from a previous review.[39] (ii) Reprinted with permission from ref 19. Copyright 2010 American Association for the Advancement of Science. (v) Reprinted with permission from ref 21. Copyright 2010 Springer Nature. (viii) Reprinted with permission from ref 20. Copyright 2012 Springer Nature. (ix) Reprinted with permission from ref 8. Copyright 2012 Springer Nature. (x) Reprinted with permission from ref 14. Copyright 2014 Springer Nature.

the redox reactions in solution without electrode material consumption. Importantly, these noble metals are chemically inert and thus benign for cell culture, while other metals, such as Ag, are known to be cytotoxic. Their polarizable nature, however, prevents the passage of current at small electrode potentials and results in a large and capacitive impedance to the solution. Though both Au and Pt electrodes are often used as capacitive sensors,[2,8,21] they have also been used in the Faradaic regime to reduce the nanoelectrode impedance at the expense of reduced interrogation time.[13] Beyond noble metals, silicon has been used in the context of a field effect transistors[19,20] and also as a capacitive interface.[18] IrO_2, a material that has already been exploited for extracellular and implantable electrodes, has recently been tested for nanoelectrodes where its pseudocapacitance significantly reduces the nanoelectrode impedance (~10-fold improvement over Au).[14]

In the realm of microscale electrodes, nanoscale rough surfaces have been used to increase the electrodes' effective surface areas to reduce the electrode impedance. The most popular choices are nanostructured noble metals, such as Pt black,[25] due to their chemical inertness. Nanomaterials with high surface to volume ratio, like carbon nanotubes (CNTs), have also been shown to reduce impedance and improve signal amplitude.[26] The compatibility of deposition techniques and the extent of impedance improvement of such coatings in nanoelectrode geometry are open questions that need to be investigated.

2.4. Chemical Modification

Chemical modifications are usually applied on the electrodes and the substrate with the goal of improving cell viability and seal resistance. These chemical coatings interact with the membrane and membrane proteins of the cell, thereby determining the distance between the membrane and the substrate and hence the seal resistance. Early research reviewed coatings in the context of improving the seal resistance of extracellular planar electrodes;[27] later nanoelectrode works used similar coatings, including poly-D-lysine,[13] fibronectin,[8] and RGD peptides,[21] to improve adhesion of cells (Figure 5a).

In addition to these common coatings for cell culture, researchers have also exploited the idea of membrane-mimicking coatings on the electrode, aiming to fuse the electrode and cell membrane together. For instance, lipid coatings have been used in a kinked nanowire field-effect transistor (FET)[19] and a nanotube FET,[20] with the goal of facilitating the merging with the cell (Figure 5b). Three-section electrodes containing two hydrophilic ends and middle hydrophobic band (mimicking the cell membrane's lipid bilayers) have also been tested[28] (Figure 5c). However, a later study found that in order to overcome the initial barrier of unfavorable interactions, a significant force (>1 nN) needs to be exerted on the electrode, which compresses the compliant cell membrane rather than fusing the electrode into the membrane.[29]

In parallel to nanoelectrodes on a substrate or a manipulator, many works have investigated solution-suspended nanowire insertion. In these works, cellular uptake of nanowires occur either through surface modification[30] (Figure 5d) or via phagocytosis of nonfunctionalized silicon nanowires[31] (Figure 5e). These studies give hope that such methods may be extended to tethered or attached nanoelectrodes to allow for direct connection to integrated electronics.

2.5. Electroporation-Assisted Intracellular Access

Different experimental methods may be used to lower the access resistance and enable intracellular measurement. In

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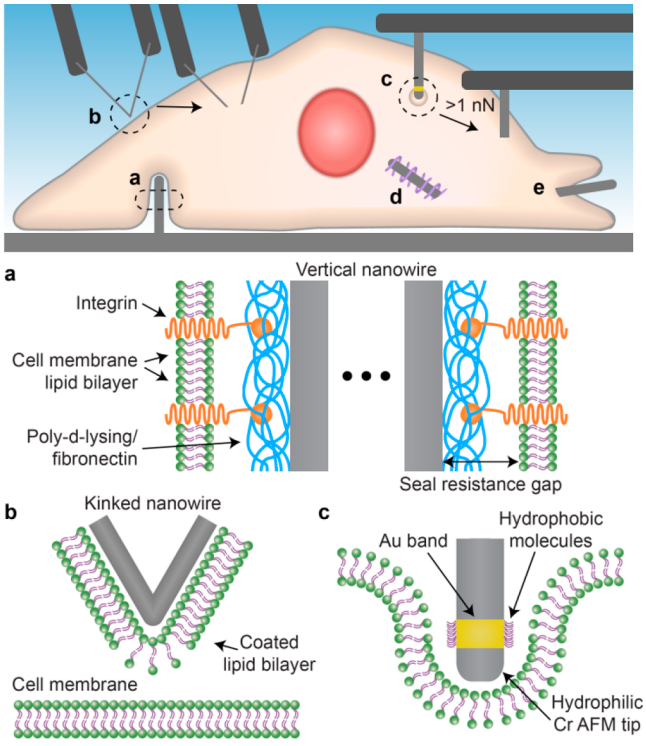

Figure 5. Chemical modifications of nanoelectrodes. (a) Nanoelectrodes are often coated with poly-D-lysing or fibronectin that in turn interact with proteins in the cell membrane (e.g., integrin) to improve adhesion and seal resistance. (b) Lipid layers may be coated onto inserted nanoelectrodes.[19] (c) Three-section electrodes containing hydrophilic ends and a hydrophobic center,[28] pushing the cell membrane until a force of >1 nN is exceeded.[29] (d, e) Suspended nanowires may pass through the cell membrane due to surface coatings[30] (d) or via phagocytosis[31] (e).

nanoelectrode studies, electroporation has been most commonly used.[2,8,13,14,16,17] In bulk electroporation that is employed in transfection applications, cells are suspended between two parallel electrodes and a large voltage (hundreds to thousands of volts) is applied to perforate the membrane. Nanoscale electrodes are able to accomplish electroporation with much smaller voltages, ~1−3 V, due to the concentration of the electric field around sharp nanoelectrode tips and the proximity of the cells to the electrodes. Unfortunately, the process has yet to be characterized in detail, and whether nanoelectrode-based electroporation leads to a single pore or a group of pores is not very well established. The effective diameter of the pore has been estimated to be 10−20 nm for ~180 nm diameter nanotubes[14] and 500−700 nm for ~1.5 μm diameter mushrooms.[17] Beyond electroporation, plasmonic optoporation,[24] overexpression,[32] and mechanical-gating of ion channels[33] have also served as pathways to bring the intracellular signal to electrodes.

During nanoelectrode-based electroporation, a significant increase in signal amplitude is observed right after electroporation pulses, indicating reduction of the access resistance for intracellular measurement. This access is transient, however (a few minutes with vertical nanopillars[2,8,17] and up to 1 h with IrO_2 nanotubes[14]): as the cell membrane recovers, the perforations in the membrane become resealed, expelling the nanoelectrodes and causing the signal amplitude to decrease.[2,8,17] Interestingly, in our most recent work, we have observed an increase in amplitude to a stable magnitude after electroporation,[2] which might indicate the possibility that after a large perforation (greater than the 150 nm nanoelectrode tip), the cell membrane may reseal around the base of the nanoelectrode, leaving the tip exposed to the intracellular solution. If such a favorable reseal can be reproducibly engineered, it may permit large amplitude and long-term recordings.

2.6. Electronics

Most nanoelectrode devices studied to date have been passive devices where the sensing component is connected via interconnects to off-device electronics.[8,13,14,18,21,24] The interconnects, usually metal traces covered with dielectric materials, often exhibit parasitic impedances comparable to or even smaller than the nanoelectrode's large impedance, causing a significant signal attenuation. Both thicker passivation and reduction in interconnect length can help to increase the parasitic impedance, but fundamental limitations exist due to fabrication capabilities and minimum device sizes/proximity to off-chip electronics.

One way to address this issue is to use on-chip active electronic components made using CMOS technology, as demonstrated in our recent CMOS nanoelectrode array (CNEA).[2] The electronics for amplification and stimulation are first fabricated using standard CMOS technology and the nanoelectrodes are postfabricated right on top of the CMOS circuit. Because the amplifier is right below each nanoelectrode, electrode-to-electronics interconnects are practically eliminated. Furthermore, the customized amplifier enables a high input impedance to be designed. In total, this strategy drastically increases the parasitic and amplifier input impedances, improving overall signal sensitivity. As discussed in the following section, the marriage between on-chip electronics and nanoelectrodes also helps to scale up the nanoelectrodes.

3. SCALABILITY

Two important features are necessary to achieve high-fidelity network-level recordings using electrode-based tools: scalable intracellular electrodes to couple to the cells and scalable electronics to record each electrode's electrophysiological signal.

3.1. Nonscalable and Scalable Electrodes

While patch clamp studies have long served as a benchmark for any electrophysiological measurements, the complexity of the patching process and the scale of micromanipulators has limited the largest setups to ~10 in parallel.[1] For the same reasons, nanoscale electrodes that need to be individually manipulated, such as kinked nanowires[19] or sharp pipets, are also difficult to scale. The development of the planar patch clamp has exceeded the throughput of the traditional patch clamp through advances in microfluidic fabrication. The challenge of building a large number of addressable microfluidic channels, however, has limited their operation to either isolated cells or parallel-ensemble circuit recording,[34] thus impeding investigations of network dynamics.

In comparison, nano- and microfabricated electrodes can be constructed reproducibly with high precision. The scalability of such electrodes is determined by the parallelization and controllability of the fabrication processes. For example, serial fabrication processes, such as focused ion beam (FIB) milling/deposition[8,24] and electron beam lithography,[13,23] are difficult to scale. Parallel fabrication techniques, on the other hand, drastically reduce fabrication time. Photolithography is the

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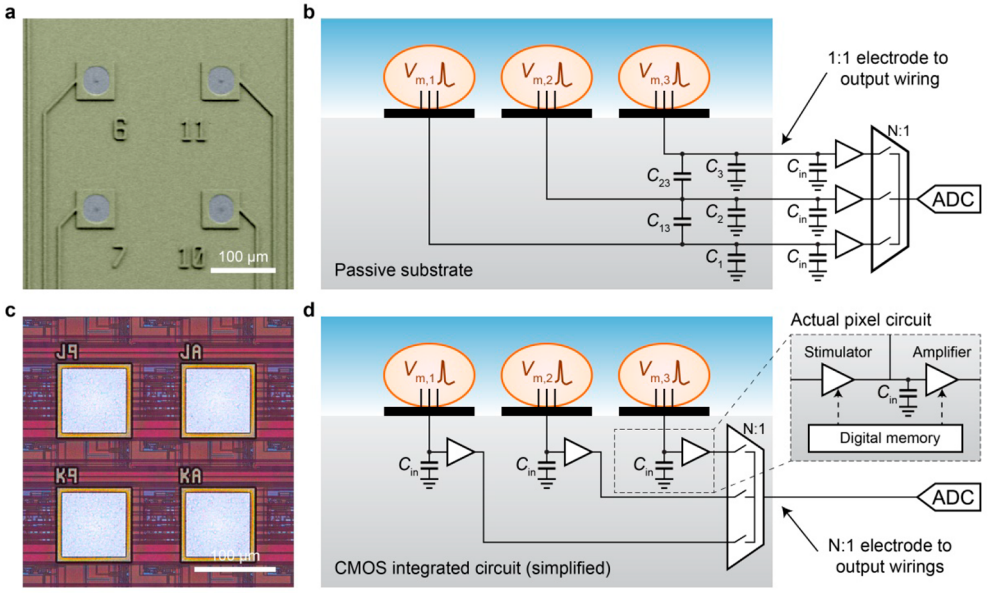

Figure 6. Advantages of electronics/electrode integration. (a, b) Stand-alone nanoelectrodes wired to off-substrate electronics suffer from parasitic capacitance (C_{1-3}) and input capacitance (C_{in}) that attenuate the intracellular signal ($V_{m,1-3}$) and cross-talk capacitance ($C_{13,23}$) that couples signals from nearby wirings; each electrode requires an off-substrate output wiring. Example shown in (a) is from the vertical nanoelectrode array (VNEA) with a 4 × 4 (16) array of electrodes.[13] Adapted with permission from ref 13. Copyright 2012 Springer Nature. (c, d) Electrodes fabricated directly on top of amplifiers within a CMOS integrated circuit eliminate parasitic capacitances and cross-talk. The number of wirings to off chip electronics can be reduced by using a N:1 multiplexer. The example shown in (c) is from the CMOS nanoelectrode array (CNEA) with a 32 × 32 (1024) array of electrodes; a 128:1 multiplexer is implemented, using 8 output wirings.[2] The pixel circuit also contains a stimulator to excite interfaced cells and a digital memory to control the operation of the stimulator/amplifier. Adapted with permission from ref 2. Copyright 2017 Springer Nature.

most commonly used parallel technique: light illumination transfers the pattern of a mask onto photoresist over the whole substrate. This method is routinely used in industry to defined sub-100 nm structures and can therefore be adapted by nanoelectrodes.[2,18,21,23] Another process that can be made parallel is the electrodeposition method, e.g., used to define Au mushroom shaped electrodes[21,32] and IrO_2 nanotubes:[14] here the geometry and location of the electrodes can be controlled via prepatterning.

3.2. Electrical Interface

Earlier electrophysiological interfaces employed off-substrate connections to interface with measurement electronics to record relatively small electrophysiological signals and stimulation circuitry to excite the cells.[35] Improvements in microfabrication techniques, mainly driven by the CMOS industry, have enabled scaling up the number of electrodes while further enabling the integration of measurement electronics on the same substrate as the electrodes.[36]

The electronics/electrode integration (Figure 6c, d) offers several advantages over stand-alone electrodes with off-substrate electronics (Figure 6a, b). First, on-substrate amplifiers eliminate attenuation of the electrophysiological signal by minimizing large lead capacitances associated with off-substrate wirings. Second, analog multiplexers can interleave data from multiple electrodes over a single wire, eliminating the need to wire each electrode off-substrate, which becomes increasingly difficult as the number of electrodes increases. Third, cross-talk between adjacent electrodes or electrodes' wirings can be minimized, improving signal-to-noise. Finally, digital circuitry located adjacent to the electrodes can enable fast, real-time control of each electrode's function.

Modern CMOS microelectrode arrays have utilized these advantages to scale up extracellular arrays to ~65,000 microelectrodes,[22,25,37,38] including on-substrate amplifiers, stimulation circuitry, and digital control/memory. The main disadvantage of these CMOS MEAs is their use of extracellular measurement and the associated signal attenuation and distortion.[12] To address this loss of signal integrity, our recent CNEA work combines intracellular access enabled by nanoelectrodes[2] with CMOS circuitry. We postfabricated, using CMOS-compatible photolithography methods, vertical nanoelectrodes directly on the surface of a CMOS integrated circuit that contains analog amplifiers, stimulation buffers, digital circuitry, and memory. This new tool is shown to be capable of intracellular recording from more than 300 cells, a substantial improvement over previous stand-alone nanoelectrode arrays.[8,13,14,18,21,24] Furthermore, network-level, subthreshold, intracellular signals were measured as well, demonstrating high-fidelity electrophysiology capability.[2] By combining scalable nanoelectrodes with scalable electronics, this line of work opens the door to high fidelity intracellular electrophysiology at a truly network level.

4. CONCLUSIONS

Electrical studies of electroactive cells and their networks are vitally important to the fields of neuroscience and cardiology. Nanoelectrodes, and their associated properties, enable large-scale, intracellular interfaces for network level electrophysiological studies. Intracellular recordings, similar in nature to the patch clamp, have been achieved by many groups through optimizations of the nanoelectrode−cell interface. Our recent CNEA work marks the first step toward combining such nanoelectrodes with integrated electronics for network-level intracellular investigations.

Looking forward, there is still much room for improvement and advancement beyond these initial demonstrations of

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nanoelectrode-based electrophysiology. First, no device thus far has been able to intracellularly probe large-scale neural networks. Recent works have demonstrated the ability to record from single mammalian neurons[13,18,24] (Table 1), however, suggesting that multisite, intracellular recording from a large number of neurons will be achievable in the near future. Second, the common use of electroporation (Table 1) to gain intracellular access has much room for improvement. This electroporation may be detrimental to many other electrogenic cells, and developments of gentler electroporation protocols or exploration of other cell−electrode coupling methods may enable safer intracellular access without affecting cell viability or function. The candidates for the latter include mechanical assistance, chemical modification[19,20,28] (e.g., hydrophobic electrode construction), and overexpression[32] or mechanical-gating[33] of ion channels.

Finally, to probe functional neuronal networks composed of thousands to millions of cells, the number of intracellular nanoelectrode sites should be increased significantly.[22,25,37,38] For nanoelectrode-based methodologies, this requires a reliable cell-to-electrode interface and corresponding scalable electronics. Furthermore, to decipher neuronal circuit function, both stimulation and recording should be possible and preferably performed simultaneously, similar to the patch clamp's voltage clamp and current clamp capabilities. Although intracellular stimulation using nanoelectrodes in neurons[13] and cardiomyocytes[2] has been demonstrated, simultaneous stimulation/recording through the same electrode has not been realized yet. With future improvements, however, nanoscale electrodes may be able to surpass the capabilities of the patch clamp technique by performing intracellular recording and stimulation at the network scale, thus enabling new fundamental studies in neuroscience and cardiology as well as associated pharmacological investigations.

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Notes

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