

Chapter 2

Nanoscale Field-Effect Transistors for Minimally Invasive, High Spatial Resolution, and Three-Dimensional Action Potential Recording

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2.1 Introduction

This chapter is devoted to the development and application of nanoscale field-effect transistors (FETs) for neural and cardiac activity recording. Compared to optical methods, the electrical recording of action potentials has high signal-to-noise ratio (SNR) and temporal resolution. But the need for electrodes limits its spatial resolution and also poses perturbation on the biological system under investigation. One way to overcome these problems is to develop electrical recording devices with nanometer size and high-density scaling-up ability. Microfabricated metal electrodes can be readily patterned into arrays, but it is hard to decrease the size of these electrodes to nanometer scale because of the necessity to ensure a reasonable impedance value at the electrode/electrolyte interface for sufficient SNR. Field-effect transistors (FETs), on the other side, can sense the potential of the solution independently on the device/electrolyte interface impedance and hence allow for the miniaturization of the probes to nanometer scale, which is important for minimally invasive, high spatial resolution electrical recording and mapping of neuronal activities, as will be discussed in this chapter.

In a standard FET, the conductance of the semiconductor channel between the source (*S*) and (*D*) drain electrodes is modulated by a third gate (*G*) electrode capacitively coupled through a thin dielectric layer to the semiconductor. In the case of a p-type semiconductor, applying a positive gate voltage depletes majority of charge carriers (positive holes) and reduces the conductance, whereas applying a negative gate voltage leads to an accumulation of carriers and a corresponding increase in conductance (Fig. 2.1) [1, 2]. If the FET is immersed into an electrolyte solution, the solution can act as analog of metallic gate electrode in the conventional FET

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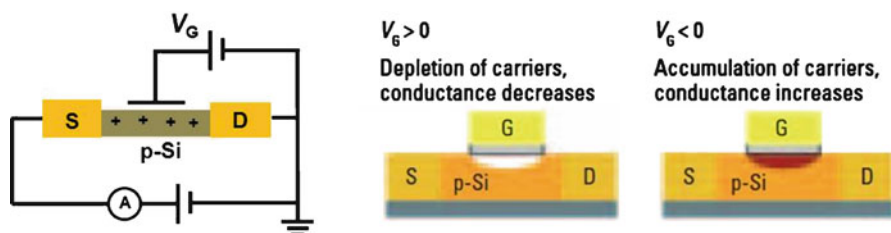


Fig. 2.1 FET voltage sensors. S, source; D, drain; and G, gate electrodes; V_G , gate voltage. Reproduced with permission from [2], © (2006) American Chemical Society

configuration and thus is termed a water gate. The dependence of the conductance on gate voltage makes FETs natural candidates for electrically based voltage sensing.

There are various ways to make the nanoscale FETs (nanofETs) to fulfill the purpose of voltage sensing and action potential recording with minimal invasiveness and high spatial resolution. In this chapter, we choose to mainly talk about the nanofETs which use freestanding, one-dimensional (1D) semiconducting nanomaterials, such as silicon nanowires (SiNWs), as the active channels of the FETs. Compared to their planar counterparts, the 1D nanoscale morphology of nanowires leads to depletion or accumulation of carriers through the entire cross section of the device from the potential change on the surface of a nanowire versus only a shallow region near the surface of a planar device. This leads to higher sensitivity for voltage sensing by using nanowire FETs [3]. Furthermore, the bottom-up paradigm used to fabricate nanowire FETs allows nanoscale FETs to be fabricated on nearly any type of surface, including those that are typically not compatible with standard CMOS processing, such as flexible plastic substrates. And it also enables the fabrication of flexible, three-dimensional (3D) freestanding devices, which can be utilized as the macroporous scaffold for synthetic tissue constructs and used for neuronal and cardiac activity monitoring throughout the 3D cellular networks inside the constructs, capabilities that are hard to achieve with conventional probes or even recently developed flexible electronics. Finally, the structure, morphology, physical properties, and corresponding functions of semiconducting nanowires can be readily controlled by encoded synthesis, which makes them ideally suited for hierarchical design of various extracellular and intracellular probes, as will be talked about later in this chapter.

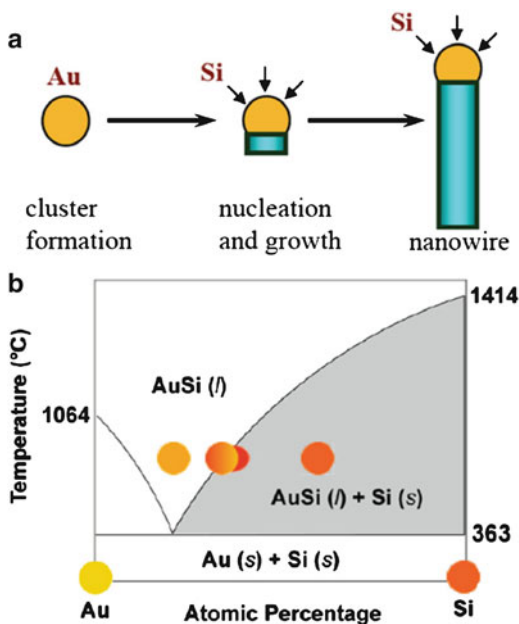
In the next section, we will briefly go over some basics related to nanowire FET, including the general method for nanowires preparation and nanowire FETs fabrication. Then we will discuss the development and application of novel SiNW FET devices as high spatial resolution and minimally invasive extra- and intracellular action potential recording probes. Lastly, we will talk about the recent progress of developing 3D macroporous, freestanding nanofETs for 3D electrical interfacing with living biological systems, such as synthetic neural and cardiac tissue constructs.

2.2 Nanowires and Nanowire FETs

Semiconductor nanowires exhibit a conductivity change in response to variations in the electric field or potential at their surface, which can be configured as FET channels and used for potential sensing. SiNWs are one of the best characterized examples of semiconducting nanowires which can be prepared as single crystal structures with diameters as small as 2–3 nm [4]. They can be prepared as p- or n-type materials and configured as FETs that exhibit electrical performance characteristics comparable to or better than those achieved in the microelectronics industry for planar silicon devices. Semiconductor nanowires are generally synthesized by employing metal nanoclusters as catalysts via a vapor–liquid–solid (VLS) process [5, 6]. In this process, the metal nanoclusters are heated above the eutectic temperature for the metal–semiconductor system of choice in the presence of a vapor-phase source of the semiconductor, resulting in a liquid droplet of the metal/semiconductor alloy. The continued feeding of the semiconductor reactant into the liquid droplet supersaturates the eutectic, leading to nucleation of the solid semiconductor. The solid–liquid interface acts as a sink causing the continued semiconductor incorporation into the lattice and, thereby, the growth of the nanowire with the alloy droplet riding on the top [6] (Fig. 2.2).

The gaseous semiconductor reactants can be generated through decomposition of precursors in a chemical vapor deposition (CVD) process. In CVD-VLS growth, the metal nanocluster serves as a catalyst at which site the gaseous precursor decomposes, providing the gaseous semiconductor reactants. In the case of SiNWs growth

Fig. 2.2 Schematic of VLS growth of SiNWs. (a) A liquid alloy droplet AuSi is first formed above the eutectic temperature (363 °C) of Au and Si. The continued feeding of Si in the vapor phase into the liquid alloy causes oversaturation of the liquid alloy, resulting in nucleation and directional nanowire growth. (b) Binary phase diagram for Au and Si illustrating the thermodynamics of VLS growth. Reproduced with permission from [6], © (2006) IOP Publishing



(Fig. 2.2), silane (SiH_4) and Au nanoparticles are normally used as the precursor and catalysts, respectively [6]. The diameter of the nanowire is determined by that of the starting nanocluster in CVD-VLS growth, and nanoclusters with diameters down to a few nanometers are now commercially available. The crystallographic orientation of the nanowires during VLS growth is chosen to minimize the total free energy, as the process is thermodynamically driven. In reality, it is determined by the diameter and growth condition such as temperature and pressure.

The VLS process offers one key advantage compared with other approaches such as vapor–solid growth or solution-based liquid–solid growth, in that the heterostructures can be achieved at the individual device level in a controlled fashion. Axial heterostructures, in which sections of different materials or same materials with different dopant level of the same diameter are grown along the nanowire axis, can be readily realized by switching the reactant during the growth [7]. By doing VLS first and then vapor–solid growth which homogeneously deposits a conformal shell on the preformed nanowire surface versus reactant addition at the nanoparticle catalyst, radial heterostructures with core/shell and core/multi-shell can form along the radial direction [8]. 3D hierarchical branched nanowire structures with same or different materials for backbones and branches can be realized by multistep VLS growth [9]. In addition, the morphology of nanowires can also be controlled by adding disturbance during VLS growth to obtain, for example, the kinked nanowires [10]. These added controls over morphology, composition, and structure can play a critical role in creating novel nanoscale FET devices for extra- and intracellular action potential recording.

To make nanowire FET devices, the nanowires can be randomly dispersed from solution by drop casting or assembled by using contact printing onto substrates such as SiO_2/Si wafers. *S* and *D* metal electrodes are then defined on nanowires by using photolithography or electron-beam lithography. To get nanowire FETs with good electrical performance, the metal used as *S* and *D* needs to match the work function of the semiconducting materials, same as in the conventional planar devices. Besides, for the application of action potential recording, other considerations, such as the metal corrosion in electrolyte and biocompatibility issue, need to be taken as well. For SiNWs, palladium (Pd) with thin layer of titanium (Ti) as adhesion has been shown as a good candidate for *S* and *D* electrodes. Lastly, passivation on the metal connection lines using insulating layers such as SiN_x or SU-8 is needed for the nanowire FET potential sensing. In this chapter, the diameter of SiNWs used in action potential recording probes is normally in the range of 10–100 nm, and the channel length of the nanowire FETs, which is the nanowire length between the *S* and *D* electrodes, is in the range of 50–2,000 nm. Figure 2.3a shows the scanning electron microscope (SEM) image of a typical SiNW FET. An example of conductance versus water-gate voltage traces for three representative p-type SiNW FETs is shown in Fig. 2.3b, where all three FETs exhibit a conductance decrease in response to the increase of the solution potential [3]. For voltage sensing with FETs, the recorded voltage values are normally calibrated from the conductance change and sensitivity of the FETs, and the sensitivity can be modulated by changing the water-gate offset.

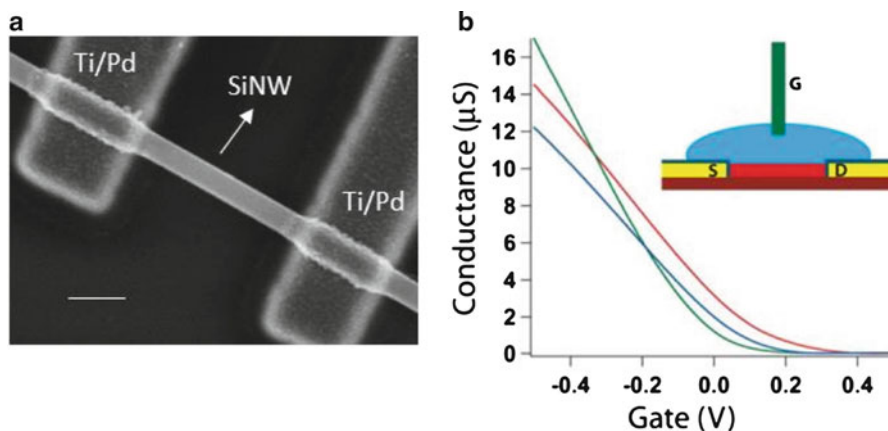


Fig. 2.3 SiNW FET voltage sensors: (a) SEM image of a SiNW FET. Here the *S* and *D* electrodes are made of palladium with titanium as adhesion layer. Scale bar is 100 nm. (b) Conductance versus water-gate voltage traces for three representative p-type SiNW FET devices. *Inset*: scheme representing experimental setup, (red) nanowire, (yellow/navy) passivated contact electrodes, (blue) electrolyte solution, and (green) Ag/AgCl reference/gate electrode [3]

2.3 Extracellular Recording with Nanoscale FETs

The potential change outside of excited cells associated with the transmembrane change can be recorded to monitor the electric activity of the cells. Compared to the intracellular electrical recording, which normally places the probes inside the cells, extracellular recording is much less invasive and provides ready access to simultaneous multisite recording. To date, the most widely used technique for extracellular recording of neuronal activities is the multielectrode arrays (MEAs) [11]. The size of the metal electrodes used in MEAs is normally in the range of 5–100 μm. This size ensures a reasonable impedance value at the electrode/electrolyte interface and gives sufficient SNR for action potential detection. With the use of FETs, the size of the extracellular recording devices can be decreased to the nanometer scale. This increase in spatial resolution will allow for the recording from small size critical subcellular structures, such as axons and dendrites of neurons, which is hard to achieve with MEAs. In MEA recording, the large size of metal electrodes also makes cell-to-electrode registration challenging because the measured signals for a given electrode typically are due to contributions from several nearby cells. As a result, identification of specific cellular signals generally requires complicated post-processing such as the spike sorting. With much smaller size, the nanoscale FET recording devices enable easy and accurate cell-to-electrode registry by decreasing the probability of coupling to multiple cells. Besides, higher-density arrays can be made from nanoscale FETs, which can increase the spatial resolution in neural activity mapping.

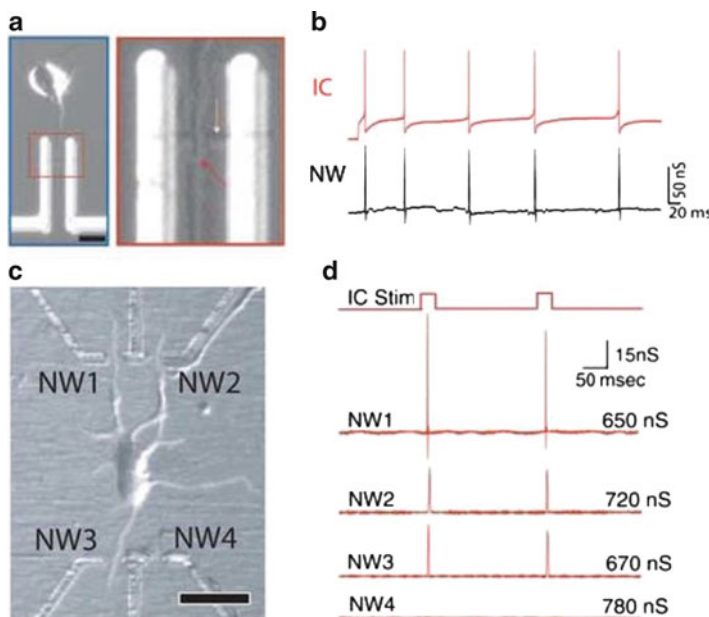


Fig. 2.4 Extracellular action potential recording from cultured neurons. (a) *Left*: optical image of a cortical neuron aligned across a SiNW FET; scale bar is 10 μm . *Right*: high-resolution image of the region where axon (red arrow) crosses a SiNW (yellow arrow). (b) Red trace: intracellular potential of a cortex neuron (after 6 days in culture) recorded by glass micropipette during stimulation; black trace: time-correlated signal from axon measured using a p-type SiNW FET. (c) Optical image of a cortical neuron interfaced to three of the four functional SiNW FETs in an array; scale bar is 20 μm . (d) Trace of intracellular current stimulation by a glass micropipette in the soma and the resulting electrical responses from the four SiNW FETs in (c). NW1~NW4 means silicon nanowire 1~4 used in the four FETs [12]

2.3.1 Extracellular Recording from Cultured Cells

The cellular and subcellular interfaces between FETs and living excitable cells can be realized by either directly culturing cells on top of the FET devices or culturing cells on a separate elastic substrate, such as polydimethylsiloxane (PDMS) sheet, and then manually bringing the cell sheet and devices chip together for extracellular recording. For direct culturing, surface patterning of polylysine can be used to guide the growth of neuron cells, including the cell body and neurites, to ensure a high yield of neuron/FET junctions and hence an efficient interfacing. With this method, SiNW FETs have successfully recorded extracellular action potential signals from neuron axons and dendrites, where the signal was in good temporal correlation with the intracellular action potentials recorded by a glass micropipette (Fig. 2.4a, b). This direct correlation indicates that the depolarization of cell membrane during action potential firing results in negative charging of the extracellular space around

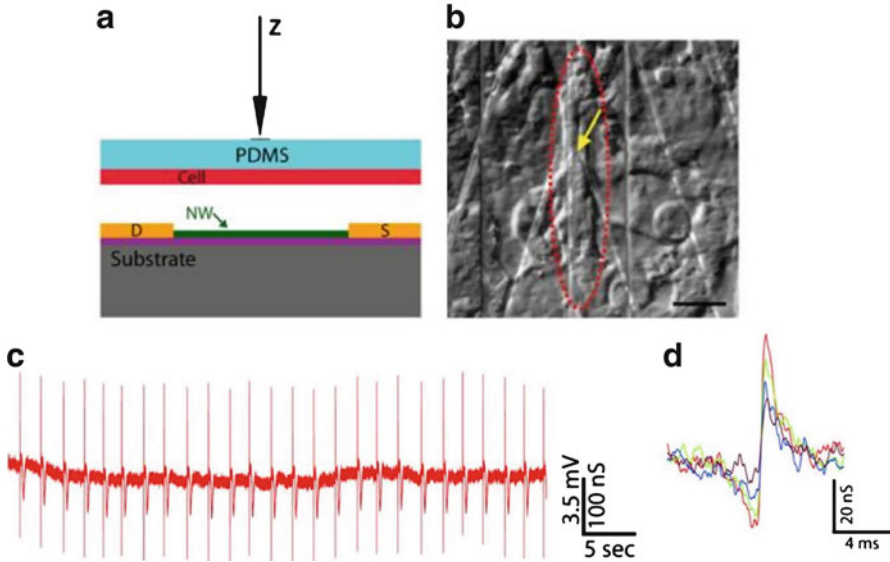


Fig. 2.5 Building interface between SiNW FETs and spontaneously firing cardiomyocytes. (a) Schematic illustration of manipulating the PDMS/cell substrate to make the interface with an NWFET device. NW means nanowire, same as below. (b) Distinct patch of beating cells (red dashed oval) over a SiNW device (yellow arrow). Scale bar is 20 μm . (c) A representative trace recorded by a SiNW FET from a spontaneously firing cardiomyocyte at displacement value close to cell failure. (d) High-resolution comparison of single spikes recorded with increasing displacement values (from purple to red) [13]

the nanowire, which is consistent with the fact that the membrane expresses a relatively high density of Na^+ ion channel. Multiplexed recording from different dendrites and axon of a single neuron was also realized by using high-density SiNW FETs array (Fig. 2.4c, d), which highlighted the advantage of higher spatial precision and resolution for the nanoscale-size SiNW FET probes. For the recording with SiNW FETs, the active junction area for nanowire/axon interfaces was $0.01\text{--}0.02\ \mu\text{m}^2$, which is at least two orders of magnitude smaller than microfabricated metal electrodes and previous planar FETs [12].

The second method of interfacing nanoFETs with living cells allows for separate design and optimization of the nanoFETs array and cell culture such that the two key components are brought together under precise manipulation only during the final measurement phase. Compared to direct culture, this approach enables people to identify, register to and record from specific cellular and subcellular regions with respect to nanoFET devices, and carry out multiplexed recording from well-defined multicellular configurations with overall subcellular resolution [3]. In addition, with this approach, the amplitude and SNR of the recorded signals can be tuned by manually adjusting the coupling strength between the cells and nanoFETs. As shown in Fig. 2.5, embryonic chicken cardiomyocytes were cultured on 100–500 μm thick, optically transparent and flexible PDMS pieces to form cell monolayers. By bringing the

cells closer to the SiNW FET devices, the amplitude of recorded extracellular action potential spikes can reach a value of 10.5 mV from ~ 1 mV and a maximum SNR of 25 before making irreversible changes and cessation of the spontaneous cell beating [13]. It should be noted that within this range of PDMS/cell displacement value, the spike amplitude changes are reversible, and the SiNW FET/cell interface is stable for different displacements of the cells toward the devices. The enhanced signal amplitudes can be attributed to a decrease in the gap between the cell membrane and SiNW FET devices, although further studies will be needed to quantify such junction changes.

This new interfacing strategy can also be used for multiplexing measurements, as different devices can all form tight junctions with the cells and hence simultaneously give signals with high SNR. The time shift between devices derived from the cross-correlation analysis of data traces recorded in this manner provides information about the action potential propagation direction and speed. The flexibility of interfacing with specific cellular areas for high-density multiplexed recording using SiNW FETs represents a powerful platform to study the effects of cell monolayer inhomogeneity on action potential propagation, enabling both intra- and intercellular propagation to be characterized in details for well-defined cellular structures.

The small size of nanoFETs allows for more localized and higher spatial precision recording than achievable with other larger size detectors, as reflected by the different signal shapes. As shown in Fig. 2.6, in the extracellular recording from spontaneously firing chicken cardiomyocytes, the signal recorded by the a SiNW FET with ~ 30 nm diameter and ~ 2.3 μm channel length has a peak-to-peak width of 760 ± 40 μs . This value is smaller than $1,310 \pm 40$ μs obtained from a graphene FET (Gra-FET) which uses graphene of 20.8 $\mu\text{m} \times 9.8$ μm large as the active channel for FET (the Gra-FETs were fabricated side by side on the same chip with the SiNW FETs) [14]. This result indicates that the signals recorded with the large Gra-FET (Gra1-FET in Fig. 2.6) do not represent a localized detection but rather an average of the extracellular potential from different parts of the beating cell or even from different cells, which caused the broadening of the peak. However, the signal recorded by another Gra-FET (Gra2-FET in Fig. 2.6) with active channel of 2.4 $\mu\text{m} \times 3.4$ μm , which is 16 μm away from the SiNW FET, gives peak-to-peak width of similar value, 730 ± 40 μs , although the SiNW FET has $\sim 100\times$ smaller active detection area [14]. This can be explained by the micrometer-scale channel length of the SiNW FET detector. Although the detection can be localized in nanometer scale in the radial direction, there will still be average from the micrometer long axial detection, which leads to the broadened peaks detected by the SiNW FET compared to the intrinsic values expected for sodium ion channels.

To make real “point-like” nanoscale detectors, the active channel length needed to be shrunk down to the size comparable to the nanowire diameter. One way of making this short-channel nanowire FET is to put the source and drain electrode close to nanoscale distance. However, these metal electrodes will physically limit cell access and also electrostatically screen the active nanowire channel, making it less sensitive to the potential change of the solution around it [15]. With VLS grown nanowires, the “point-like” ultrasmall detector can be realized in a unique way of

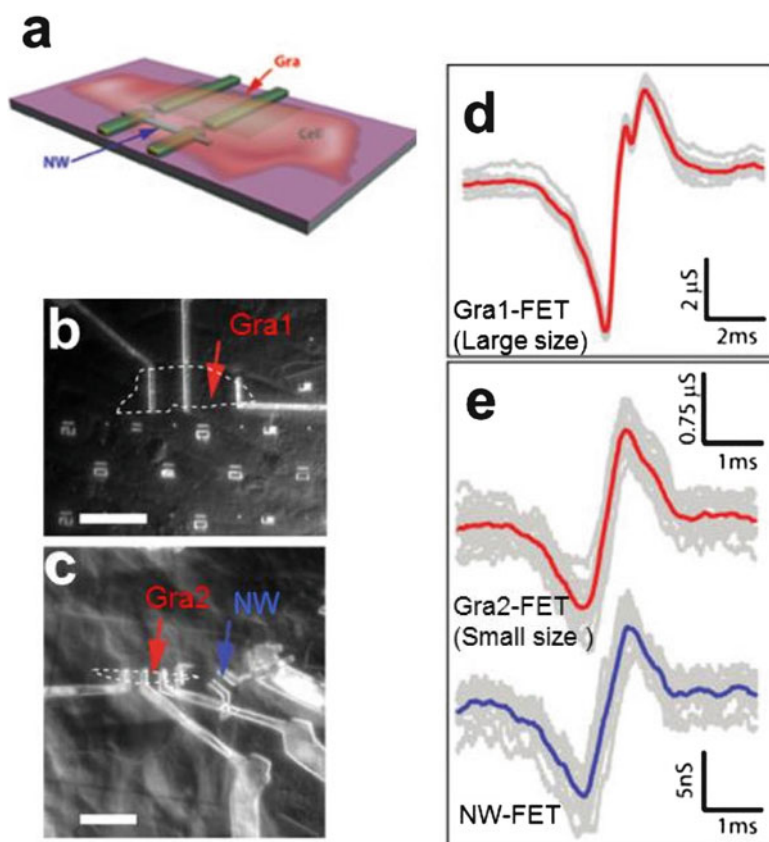


Fig. 2.6 Extracellular action potential recording from FET probes with different size of active channels. (a) Schematic illustrating the chip design incorporating graphene and SiNW FET devices. (b) Optical microscope image of PDMS/cells interfaced with large flake graphene FET. Scale bar is 30 μm . (c) Optical microscope image of PDMS/cells interfaced with smaller flake graphene FET and SiNW FET. Scale bar is 13.6 μm . Graphene flake outline is marked by *white dashed line*, measured graphene device is marked by *red arrow*, and measured SiNW device is marked by *blue arrow*. (d) Recorded averaged peak (*red*) and raw data (*gray traces*) for the Gra1-FET and cell in (b). (e) Thirteen raw signal peaks (*gray traces*) from the Gra2-FET (*upper data*) and SiNW FET (*lower data*) devices marked by *red* and *blue* arrows, respectively, in (c). The average of the peaks was plotted in *red* and *blue*, respectively. Reproduced with permission from [14] © (2010) American Chemical Society

dopant modulation along axial direction. By changing the dopant ratio in the feeding sources during nanowire growth, short length of lightly doped nanowire segment with two heavily doped nanowire segments connected to its two ends can be synthesized. This short lightly doped nanowire segment will be the FET active channel and two heavily doped arms function as nanoscale *S/D* electrode arms. Metal interconnects are then placed on these two arms, thus ensuring an intimate contact between the cells and detectors.

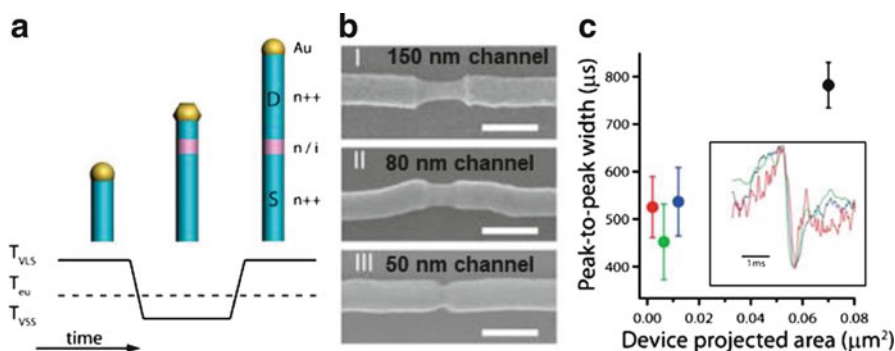


Fig. 2.7 Extracellular action potential recording with short-channel SiNW FETs. (a) Illustration of Au-nanocluster-catalyzed nanowire growth with well-controlled axial dopant profile introduced during VSS growth. (b) Short-channel n⁺⁺/i/n⁺⁺ SiNWs with channel lengths of 150, 80, and 50 nm, respectively. Scale bars are 150 nm. The Au nanoclusters were ~80 nm in diameter, and nanowires were selectively etched to reveal the active channel. (c) Summary of the peak-to-peak widths for the 150, 80, and 50 nm channel length devices. In addition, a previously published 2.3 μm channel length SiNW FET device (black) is shown for comparison [14]. Inset, an example of single peaks from each of the short-channel devices. Reproduced with permission from [16] © (2010) American Chemical Society

Except for VLS mode, nanowire growth can also use a vapor–solid–solid (VSS) mechanism; in this case, the growth temperature is below the eutectic temperature, and instead of liquid metal–semiconductor alloy droplets, a solid alloy forms and acts as the nanowire growth interface [17]. In VSS growth, the nanowire growth rate is at least 10–100 times lower than for VLS growth. By switching to VSS for the lightly doped nanowire segment (while maintaining VLS for the most part of the heavily doped nanowire arms growth to keep a reasonable growth time in total, Fig. 2.7a), short-channel nanowire devices can be realized in a way which enables control of the lightly doped nanowire channel segment on a 10 nm scale and abrupt lightly heavily doped nanowire junctions. SEM imaging of selectively etched lightly doped nanowire segments (Fig. 2.7b) showed that nanowires with channel lengths of 150, 80, and 50 nm could be synthesized using this approach [16].

Extracellular recording from spontaneously firing cardiomyocytes using SiNW FETs configured from these new short-channel SiNWs (Fig. 2.7c) yielded action potential signals with peak-to-peak widths of 520 ± 40 , 450 ± 80 , and 540 ± 50 μs for the 150, 80, and 50 nm devices, respectively [16]. These widths are smaller than the peak-to-peak widths of 750–850 μs recorded with the conventional nanowire with micrometer-scale active channels discussed above, which indicates the advantage of the recording with “point-like” detectors to avoid extrinsic temporal broadening. Interestingly, the time scale reported for Na⁺ channel conduction is about 500 μs [18], which is consistent with the peak widths measured from the short-channel SiNW FETs here. This suggests that these short-channel SiNW FET devices may be able to study ion channel on the length and time scale of single ion channel events in the future.

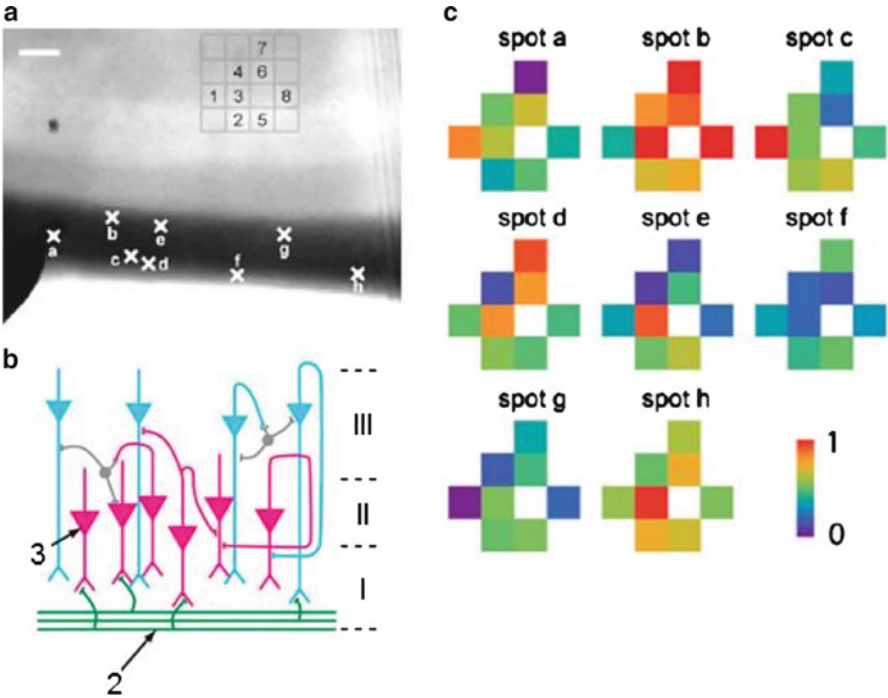


Fig. 2.8 Extracellular recording from acute brain slices with SiNW FETs. (a) Optical image of an acute slice over a 4×4 SiNW FET array. Crosses along the LOT fiber region of the slice mark the stimulation spots *a–h*. Scale bar is 100 μm. (b) Laminar organization and input circuitry of the piriform cortex (layers I–III). (c) Maps of the relative signal intensity or activity for devices 1–8 [19]

2.3.2 Extracellular Recording from Tissue Slices and Organs

In the brain, neural circuits are organized through synaptic connections into hierarchical networks operating on spatial and temporal scales that span multiple orders of magnitude. From this perspective, electrical recording with high spatiotemporal resolution from populations of neurons using nanoscale FET arrays presents unique opportunities in neural activity mapping of the brain tissue. On the other hand, electrical recording in vitro and in vivo from whole hearts is important in areas ranging from basic studies of cardiac function to patient healthcare. The flexible and transparent recording chips enabled by the bottom-up paradigm used in nanowire FETs fabrication provide a powerful way to make conformal contact to the 3D soft tissue of heart, as will be discussed in this section.

The high-resolution recording capability of SiNW FET arrays was exploited to map the neural connectivity in the olfactory cortex of a rat acute brain slice as shown in Fig. 2.8. An optical image of an oriented brain slice (Fig. 2.8a) shows the lateral olfactory tract (LOT) (*dark band*) and the pyramidal neuron layers. A schematic of the organization and circuit of the slice (Fig. 2.8b) highlights the LOT and

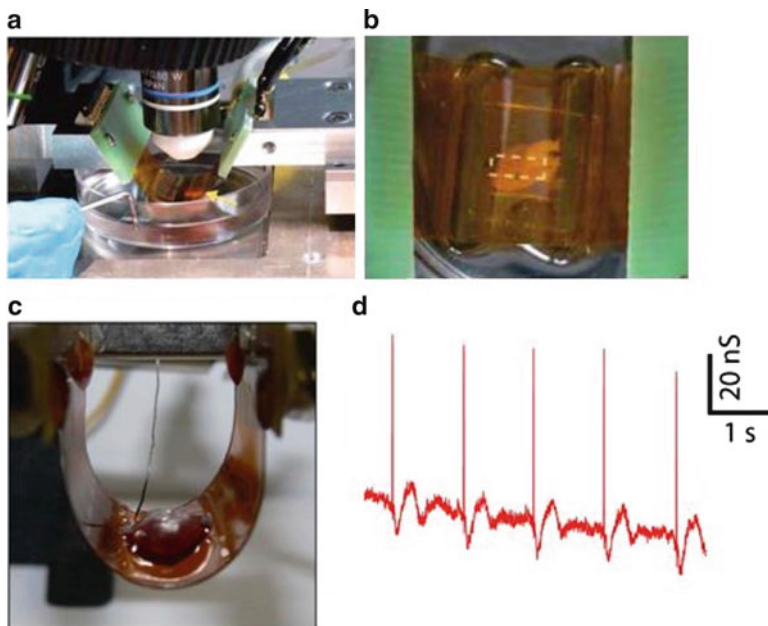


Fig. 2.9 Extracellular recording from whole hearts with SiNW FETs fabricated on flexible plastic substrate. (a) Photograph of a flexible SiNW FETs chip interfaced with a chicken heart in concave configuration. *Yellow arrow* marks the location of the heart. (b) Top-down photograph of same system, which enables overall registration between heart and lithographically defined markers on the substrate. (c) Photograph of a flexible SiNW FETs chip interfaced with a chicken heart in convex configuration. (d) A representative recorded conductance data from a SiNW FET interfacing with chicken heart in bent configuration. Reproduced with permission from [20] © (2009) American Chemical Society

synaptic connections (*layer I*) with the pyramidal cells in *layers II* and *III*, which are oriented over the SiNW FET array. Following stimulation at eight different spots or different sets of axon fibers in the LOT (a–h, Fig. 2.8a) using a metal electrode or glass micropipette, eight SiNW FETs (1–8, Fig. 2.8a) showed distinct responses in terms of the amplitude for the recorded postsynaptic signal, as shown in the 2D maps in Fig. 2.8c. These 2D maps from the SiNW FET array resolved clearly the heterogeneous activity of the neural circuit [19]. Note that the distance between adjacent devices, 60 μm , in which distinct activity was recorded is already better than the 100 μm scale resolution achieved in MEA recording of brain slices, although the mapping resolution can be substantially improved by using higher-density nanowire FET arrays.

Silicon nanowire FETs were fabricated on thin polymer substrates and interfaced to beating embryonic chicken hearts in bent chip configuration, as shown in Fig. 2.9. The use of flexible and transparent SiNW FETs chips enables simultaneous optical imaging and electronic recording. A bent device chip with concave surface facing a beating heart (Fig. 2.9a) shows that the SiNW FET/heart can be readily examined

with microscope. This allows for visual orientation of the device array to the heart and higher-resolution imaging through the transparent substrate (Fig. 2.9b). Recording from the whole heart in a convex configuration (Fig. 2.9c), where the heart/device interface is on the convex side of the bent chip, gives extracellular signals with excellent SNR (Fig. 2.9d). The signal correlates with the spontaneous beating of the heart and shows an initial sharp peak followed by a slower phase, where these two phases can be attributed to transient ion channel currents and mechanical motion, respectively [20].

The above studies on acute brain slices and whole hearts demonstrate the powerful capability of nanoFETs to interface with hierarchically organized tissue and organs with cellular and subcellular spatial resolution and submillisecond temporal resolution. The advantage of conformal interfaces with soft and irregularly shaped tissues and organs enabled by the flexible nanowire FET chips represents unique opportunities in cellular activity mapping, which demonstrates the potential of nanowire FETs as tools to understand the functional connectivity and address critical biological problems in the study of neural and cardiac systems.

2.4 Intracellular Recording with Nanoscale FETs

Intracellular recording has several advantages over extracellular recording. Firstly, it measures true transmembrane potential of the cells, instead of the derivative form of the cell transmembrane potential change reflected by the extracellular recording, which makes the extracellular signal much smaller in amplitude and distinct in shape/time scale from real action potentials. As a result, extracellular recording reflects the time of occurrence of action potentials but is not able to record action potentials with the details needed to explore the properties of ion channels. Second, intracellular recording can measure subthreshold events and DC or slowly changing potentials across the cell membrane associated with synaptic interactions, which is important for neural network activity but difficult to measure with extracellular probes. Third, intracellular recording enables clear cell-to-electrode registry. Extracellular signals often represent an average over several cells located at the vicinity of the probe. As discussed above, nanoscale extracellular probes, such as nanowire FETs, can overcome this issue to some extent by decreasing the probability of coupling to multiple cells. But there is still uncertainty when a nanoscale extracellular probe is close to the boundary between cells or interfaced with subcellular structures, such as dendrites. Intracellular recording, on the other hand, can register the position and signal to specific cell/electrode interfaces unambiguously.

The fact that a direct physical contact between probes and the interior of cells is needed for intracellular recording makes it more invasive. For example, commonly used glass micropipettes typically have open tip diameter of $\sim 0.2\text{--}5\text{ }\mu\text{m}$, which can be a substantial fraction of the cell size, and during recording, there will be mixing of cell cytosol and exogenous filling solution (through the open tip) that cause irreversible changes to cells and make long-term measurements difficult [21].

In addition, the large probe size also poses a challenge to record from small subcellular structures, such as axons and dendrites. Another limitation of conventional intracellular recording comes from its difficulty in simultaneous recordings at a large number of sites with high spatial resolution, which is faced by many intracellular recording probes, such as glass micropipettes, sharp metal electrodes, and carbon-based microelectrodes, which all need a micromanipulator for the recording.

By developing novel nanoscale probes with active FET detection elements, intracellular electrical recording with minimal invasiveness and toxicity, high spatial precision and resolution, and high-density, large-scale scaling-up capability can be realized. We will talk about the recently developed FET-based intracellular probes and their applications in intracellular action potential recording in this section.

2.4.1 Novel FET-Based Intracellular Probes

2.4.1.1 Kinked Nanowire FET Probes

The main challenge to realize the FET-based intracellular probes is to couple the active channel to the interior of cells in a minimally invasive manner. Unfortunately, the conventional FET geometry in which the active channel connected in a linear geometry to large S and D electrodes precludes insertion without disruption of cells. One efficient way of solving this challenge is to use kinked nanowires in which the voltage sensitive active channel—a lightly doped segment—is encoded synthetically at or close to the tip of the kink (Fig. 2.10a, b). The kink tip can be inserted into cells for intracellular interfacing. In this structure, two heavily doped nanowire arms of the kink serve as synthetically defined nanoscale S/D electrodes. By connecting the nanowire S/D arms with strained microscale metal interconnects, the device is bent up and forms a 3D, freestanding probe with the kink tip presented to the 3D space to access the cell interior (Fig. 2.10c, d) [22]. The heavily doped arms effectively prevent the metal electrodes from disrupting the cell. Since the synthetically defined active part of the kinked nanowire is localized at the tip region, the entire active channel of the FET can be coupled to the interior of the cell, thus ensuring highly sensitive transmembrane potential recording.

The variation of reactant pressure during the Au-nanocluster-catalyzed VLS growth of SiNWs can introduce reproducible 120° kinks at defined positions [22]. By purging the reactant for a short time and then reintroduce the reactants, the growth of the SiNWs experiences a pause and resume process. And the heterogeneous nucleation during the resume step causes a change of the growth orientation which results in a 120° kink in the nanowire. An intracellular recording probe can be this 120° kink or a 60° kink formed with two *cis*-linked kinked units (Fig. 2.10 a, b). The dopant modulation can be conducted right before or after the introduction of the kink or between the two kinks for the 60° probe to make the short active channel close to or at the topologically defined kink tip (the pink segment in Fig. 2.10a).

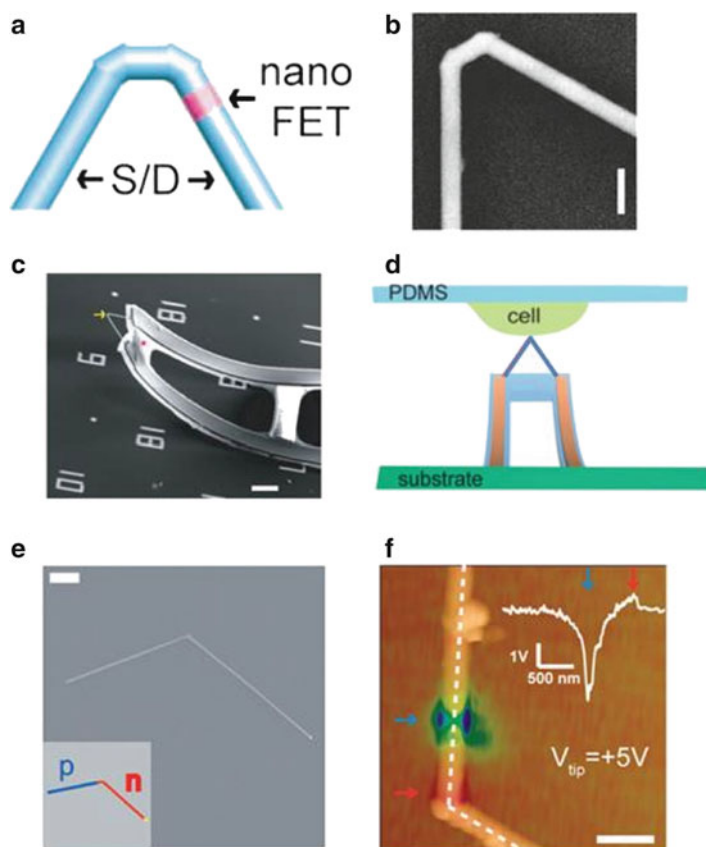


Fig. 2.10 Kinked nanowire FET probes. (a) Schematic of a kinked nanowire probe with encoded active region (pink). Blue regions are nanowire S/D. (b) SEM image of a doubly 60° kinked nanowire with a *cis* configuration. Scale bar, 200 nm. (c) A 3D, freestanding kinked nanowire FET bent-up probe. The yellow arrow and pink star mark the nanoscale FET and SU-8 (a polymer used to passivate metal electrodes), respectively. Scale bar, 5 μ m. (d) Schematic of intracellular recording using kinked nanowire probes. (e) Representative SEM image and schematic (inset) of a kinked p-n junction SiNW with 120° tip angle. Scale bar, 1 μ m. (f) Superposition of tmSGM images on AFM (atomic force microscopy) topographic images of a representative kinked p-n nanowire device under V_{tip} of +5 V. Scale bar is 0.5 μ m. The blue/red arrows indicate the p-type and n-type depletion/accumulation regions, respectively. Inset: line profile of the tmSGM signal along the white dashed lines. (a–c) Reproduced with permission from [22] © (2010) The Author(s). (d–f) Reproduced with permission from [23] © (2012) American Chemical Society

Another approach we have implemented for making active kinked nanowire probes involves incorporation of a p–n junction near the probe tip by synthesis (Fig. 2.10e) [23]. They can be realized by changing the dopant source, instead of doping ratio during the growth. The active channel is then localized at the depletion region of the p–n junction, where the theoretical width of the depletion region could be as small as 10–30 nm [24], thus allowing potentially very high-resolution

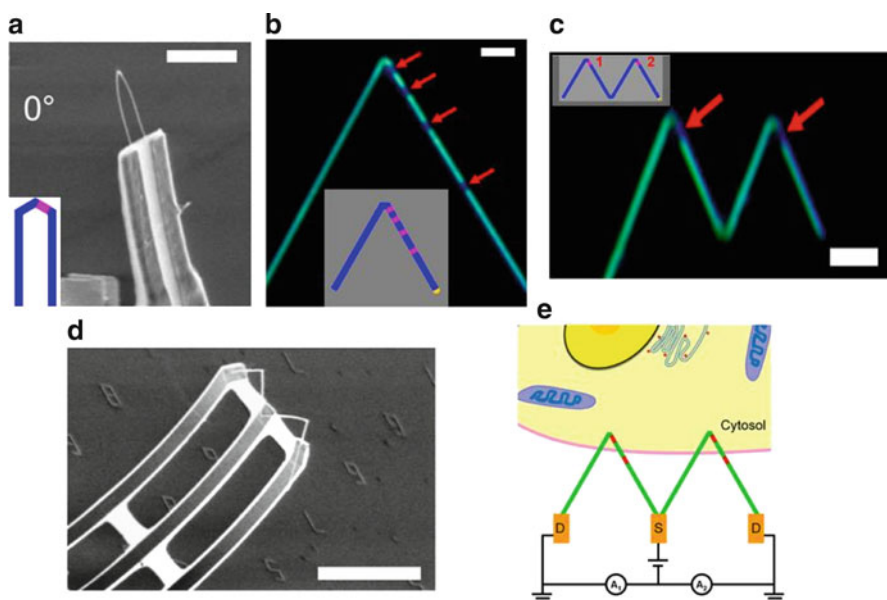


Fig. 2.11 Kinked nanowire superstructures. (a) SEM image of a 3D probe device fabricated using a 30 nm diameter U-shaped kinked nanowire. Scale bar, 3 μm . *Inset*, schematic of a U-shaped kinked nanowire with tip constructed from three 120° *cis*-linked kinks. The lightly doped n-type nanoFET element (pink) is encoded at the tip and connected by heavily doped n^{++} S/D arms (blue). (b) Dark-field optical microscopy image of a KOH-etched kinked nanowire with four nanoFETs. The dark segments correspond to the four lightly doped nanoFET elements (red arrows). Scale bar, 2 μm . *Inset*, schematic of the probe design. (c) Dark-field optical microscopy image of KOH-etched W-shaped kinked nanowire. The two dark color segments correspond to the lightly doped nanoFET elements (red arrows) near the two tips. Scale bar, 2 μm . *Inset*, schematic of the probe design. (d) SEM image of W-shaped kinked nanowire bent-up probe. Scale bar, 20 μm . (e) W-shaped kinked nanowire with multiple nanoFETs (red) illustrated as a bioprobe for simultaneous intracellular/extracellular recording. Green indicates heavily doped (n^{++}) S/D nanowire electrode arms, and red highlights the point-like active nanoFET elements. Reproduced with permission from [25] © (2013) American Chemical Society

recording. Tip-modulated scanning gate microscopy (tmSGM) measurements from a typical p–n junction kink nanowire (Fig. 2.10f) show that the synthetically defined p–n junction region near the kink exhibits a p-type gate response (because of the lower dopant concentration for the p-arm than the n-arm). The length of the p-depletion region, which defines the spatial resolution of the device, was estimated from the full width at half maximum (FWHM) of the SGM line profiles to be 210 nm [23]. This resolution is lower than the theoretical limit of 10–30 nm and represents an area where future improvements could be realized.

The strategy of encoding well-defined FET active segments into geometrically controlled nanowire superstructures for 3D, freestanding devices can be extended to prepare a variety of functional bioprobes. Zero-degree kinked nanowire probes, which have two parallel heavily doped arms in U-shape and the active nanoscale FET (nanoFET) channel located at the tip of the “U” (Fig. 2.11a), were prepared by

encoding three *cis*-kinks. The 60° V-shaped kinked nanowire probes with multiple nanoFETs encoded in series along one arm from the tip (Fig. 2.11b) can be realized by adding additional dopant modulation. Furthermore, superstructures in which two-kink nanowire devices were juxtaposed in a single W-shape with nanoFETs integrated at the tip of each of the kinked regions (Fig. 2.11c, d) were synthesized [25]. By encoding multiple nanoFETs in these complex structures and precisely controlling the probe/cell interface, these probes offer high-density multisite intracellular recording and/or simultaneous recording of both intra- and extracellular signals (Fig. 2.11e), a capability truly unique to these bottom-up nanowire structures.

2.4.1.2 Branched Intracellular Nanotube and Active Nanotube FET Probes

The kinked nanowire presents a unique design for FET-based intracellular probes. To further reduce the size of the probes and make them more amenable to large-scale high-density parallel recording, some other designs using nanotube channels to bridge between the inside of cells and FET detector elements were developed. The first design, termed as the *branched intracellular nanotube FET (BIT-FET)*, involves the use of a vertical or nearly vertical electrically insulating SiO₂ nanotube which is integrated on top of the FET channel (e.g., a SiNW channel). After the nanotube tip penetrates the cell membrane, the cytosol fills in the nanotube and gates the underlying FET, thus enabling the recording of the intracellular transmembrane potential change or action potentials (Fig. 2.12a) [26]. This BIT-FET design has several unique advantages: (1) It uses the tip of controlled diameter nanotube to interface to and probe intracellular regime, and together with an “impedance-free” FET detector, it allows for much smaller probe size, thus adds the capability for interfacing with small subcellular structure such as neuronal dendrites. (2) This design is compatible with the large-scale high-density planar nanoFET arrays, which provides a powerful way for parallel recording from large numbers of sites with spatial resolution that exceeds other probes.

The BIT-FET device was realized using bottom-up synthesis together with more conventional top-down processing, which enables an easy control over the critical probe dimensions. In short, germanium nanowire (GeNW) branches were grown on top of SiNWs, coated with a conformal, controlled thickness SiO₂ layer by atomic layer deposition (ALD), followed by selective removal of the topmost part of the SiO₂ shell and etching of the GeNW core to yield the a hollow SiO₂ nanotube on the SiNW FET as shown in the inset of Fig. 2.12b [26]. This design and fabrication scheme is not limited to SiNW FETs but can be used on any other nanoFETs including the planar ones. The etching step used to remove the upper portion of SiO₂ results in a controlled taper at the tip due to isotropic etching of the SiO₂ shell, which is particularly advantageous for further decreasing probe size.

The BIT-FETs respond selectively and with high sensitivity to the potential change of the solution inside the nanotubes rather than that outside and thus meet the requirements for intracellular recording outlined schematically in Fig. 2.12a. In terms of temporal resolution, modeling shows that the BIT-FETs with typical

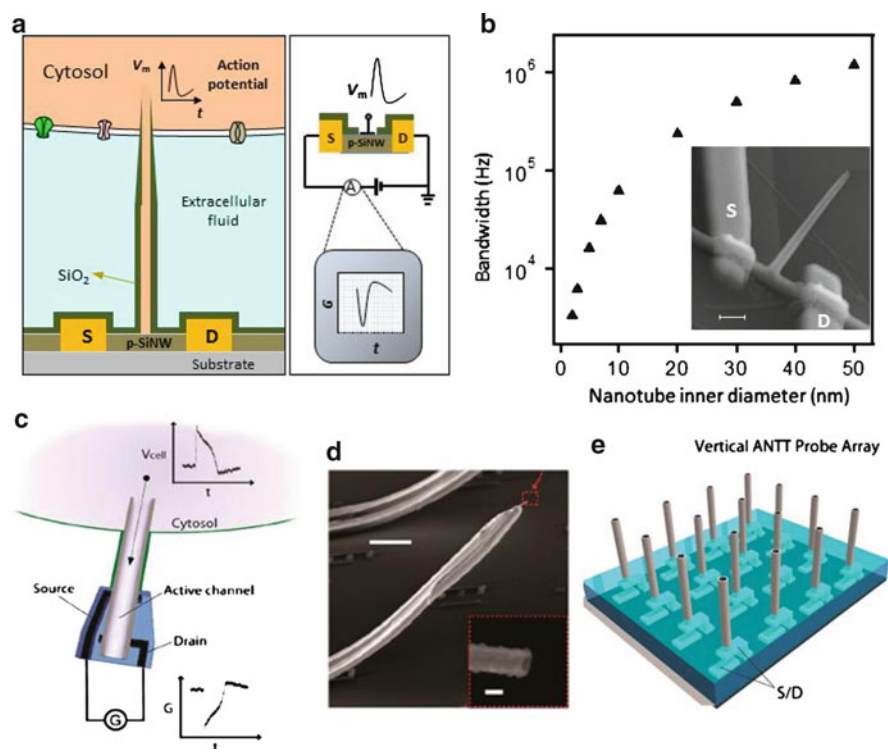


Fig. 2.12 Nanotube coupled FET probes. (a) Schematic illustrating the working principle of the BIT-FET. (b) Calculated bandwidth of the BIT-FET device versus the inner diameter of the nanotube for fixed nanotube length of 1.5 μ m. *Inset*, SEM image of a BIT-FET device. Scale bar, 200 nm. (c) Schematic illustration of the working principle for the ANTT probe. (d) SEM image of an ANTT probe. Scale bar, 10 μ m. *Inset*, zoom of the probe tip from the dashed red box. Scale bar, 100 nm. (e) Schematic of chip-based vertical ANTT probe arrays for enhanced integration. (a, b) Reproduced from [26] © (2012) The Author(s). (c–e) Reproduced with permission from [27] © (2012) American Chemical Society

nanotube dimension (inner diameter, 50 nm; SiO₂ wall thickness, 50 nm; length, 1.5 μ m) have a bandwidth in the MHz scale, which is higher than necessary for recording the neuronal action potentials. The bandwidth decreases with decreasing nanotube size but can still maintain a ≥ 6 kHz value for nanotube inner diameters as small as 3 nm according to the modeling [26] (Fig. 2.12b).

The nanotube used to couple to the cell interior can also act as the active channel of the FET detector as shown schematically in Fig. 2.12c. In this alternative nanotube-based intracellular probe design, the S/D electrodes are fabricated on one end of the nanotube while leaving the other end free for cell membrane penetration. The cytosol filling the nanotube after membrane penetration can gate the FET from inside the nanotube. This design is termed as *active silicon nanotube transistor* (ANTT) [27]. The ANTT probes were realized by synthesizing Ge/Si core/shell

nanowires and then etching away the Ge core with H_2O_2 to leave a Si nanotube. Similar to the kinked nanowire probes, strained metal electrodes are used to lift up the nanotube FET and make it accessible to cells (Fig. 2.12d). The use of microscale metal electrodes to orient the ANTT probe limits its application in large-scale high-density multisite recording. However, this limitation could be overcome in the future by preparing vertical nanotube FET arrays in a manner similar to work on vertical nanowire FETs (Fig. 2.12e) [28].

2.4.2 Intracellular Action Potential Recording with FET-Based Probes

The cell membrane penetration and high-resistance sealing between the membrane and nanoprobe are critical for faithful, stable, and minimally invasive intracellular recording. Mechanical suction or insertion has been used successfully by many conventional recordings. In the studies of the above nanoFET probes, a different approach using phospholipid bilayer modification was applied to facilitate the cell membrane penetration which shows advantages compared to the mechanical method. As shown in Fig. 2.13, when bringing a dissociated HL-1 cell (a mouse cardiac muscle cell line) into contact with a kinked nanowire probe, where the intracellular potential of the cell was clamped at a certain value with a glass micropipette, the probe modified with the phospholipid bilayer showed clear change in calibrated potential to a value close to the intracellular potential of the cell, which is indicative of the cell membrane penetration. But the unmodified probes exhibited only baseline fluctuations ($<\pm 1$ mV) [22]. These results show that the phospholipid bilayer modification helped the cell membrane penetration by the kinked nanowire FET probe, even when there is no external force applied. While there is no detailed study on this membrane penetration process or the nature of the membrane/probe interface, a speculation of biomimetic lipid fusion [29] was proposed to play an important role. The fusion of the lipid bilayer on the probe with the cell membrane could result in a biomimetic and spontaneous membrane penetration by the kink tip and also a stable, high-resistance probe/membrane seal, which will be important for long-term and stable intracellular recording.

With phospholipid bilayer modification, kinked nanowire FET probe, BIT-FET, and ANTT recorded intracellular action potentials successfully from spontaneously firing cardiomyocyte cells cultured on the PDMS sheets in a setup as shown schematically in Fig. 2.5a. Representative data from a BIT-FET recording shows that approximately 45 s after gentle contact of the cell with the device, there was a dramatic change from extracellular spikes to intracellular action potential peaks with a concomitant baseline shift of approximately -35 mV (Fig. 2.14a). Because the SiNW used in this BIT-FET is p-type, the recorded intracellular conductance peaks were inverted, although the calibrated potential for these very stable peaks has the standard polarity, shape, amplitude, and duration for intracellular cardiac action potential. These intracellular action potential peaks show five characteristic

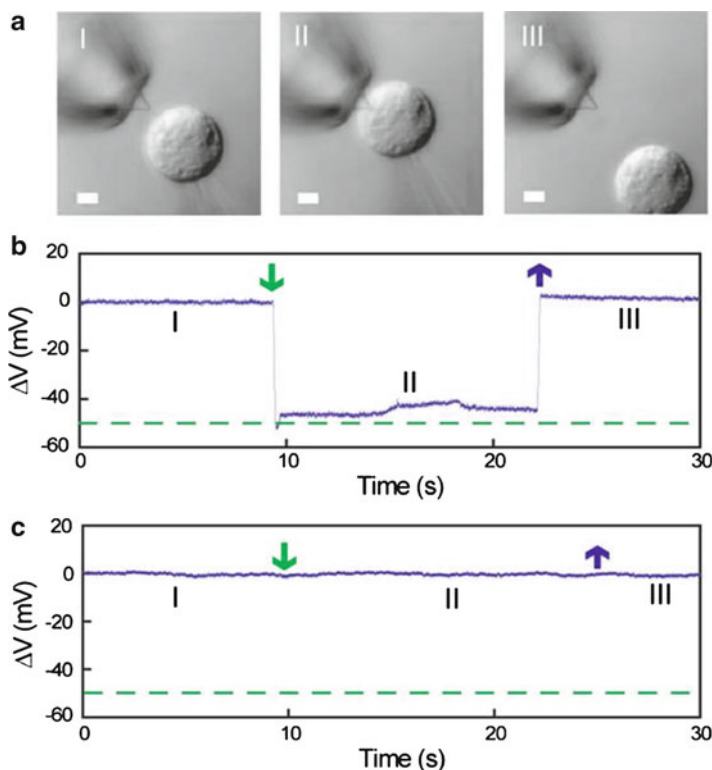


Fig. 2.13 Cellular entry by a kinked nanowire FET probe. (a, b) Differential interference contrast (DIC) microscopy images (a) and electrical recording (b) of an HL-1 cell and 60° kinked nanowire probe as the cell approaches (I), contacts and internalizes (II), and is retracted from (III) the nanoprobe. A glass micropipette was used to manipulate and voltage clamp the HL-1 cell. The dashed green line corresponds to the micropipette potential. Scale bars, 5 μm . (c) Electrical recording with a 60° kinked nanowire probe without phospholipids surface modification. Green and blue arrows mark the beginnings of cell penetration and withdrawal, respectively. Reproduced with permission from [22] © (2010) The Author(s)

phases of a cardiac intracellular potential, including (I) resting state, (II) rapid depolarization, (III) plateau, (IV) rapid repolarization, and (V) hyperpolarization (Fig. 2.14b) [26].

In the above action potential recording, and other ones using lipid modified nanoFET probes, the transition from extra- to intracellular signals, which is indicative of the cell membrane penetration, occurs without application of external force. This is consistent with the phospholipid-assisted spontaneous, biomimetic cell membrane penetration. And we believe that the small probe sizes can be beneficial for the lipid fusion and biomimetic cell membrane penetration to happen. As a result of this biomimetic penetration, a stable, high-resistance sealing between probes and cell membrane forms, as reflected by the full amplitude of the action potential signals, ~75–100 mV [26], which is recorded without the need for circuitry to

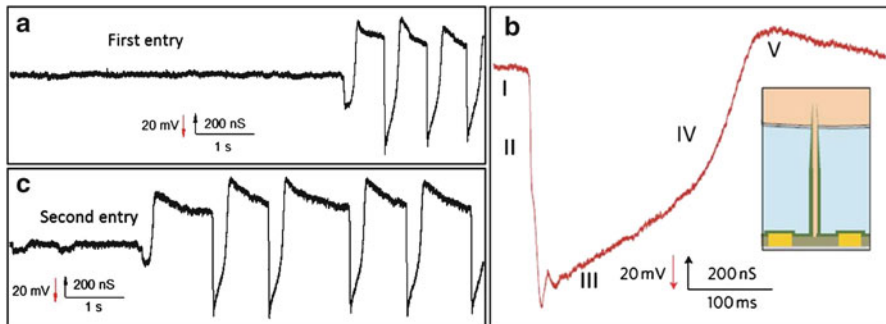


Fig. 2.14 Intracellular action potential recording by a BIT-FET. (a) Representative trace (conductance vs. time) reflecting the transition from extracellular to intracellular recording. (b) Magnified view of an intracellular peak. *I–V* correspond to the five characteristic phases of a cardiac intracellular potential. *Inset*, schematic for BIT-FET intracellular recording. (c) Trace corresponding to the second entry of the BIT-FET nanotube at approximately the same position on the cell as in (a). Reproduced from [26] © (2012) The Author(s)

compensate for putative probe/membrane leakage. In fact, control experiments carried out without phospholipid modification on the BIT-FETs required external forces to achieve the transition to intracellular action potential signals, which proved the critical role of lipid modification in this spontaneous cell membrane penetration.

Another unique characteristic of the nanoFET recording, which distinguishes them from conventional intracellular probes, is that they can be retracted from the cell and reenter approximately the same position on the same cell to record intracellular action potentials for multiple times without affecting the cell (Fig. 2.14c) [26]. This capability, a cooperative result from the small size of the nanoFET probes and the use of lipid modification, allows for long-term stable recording on scale of hours and demonstrates the reliability, robustness, minimally invasiveness of the nanoFET recording.

Simultaneous, multisite intracellular recording of action potentials from both single cells and cell networks has been demonstrated by interfacing the independently addressable nanoFET probes with cells [26, 27]. The use of phospholipid modification is especially advantageous for multiplexed recording in that it can help to achieve stable, tight sealing between multiple nanoprobe and a cell membrane (or membranes of multiple cells) at the same time. Different nanoFET probes may penetrate the cell membrane at different time, but eventually all of them yield stable, full-amplitude action potential recording thus demonstrating the possibility of large-scale parallel measurements for neural and cardiac activity mapping.

Due to their potential in high spatiotemporal resolution and large-scale brain activity mapping, nanoscale tools have received considerable attention [30]. The small size of the FET-based nanoprobe discussed here not only makes it possible for an unprecedented high-density device arrays which are critical for cellular and even subcellular resolution mapping but also yields minimal perturbation of cells and/or tissues being studied. We believe that these nanoprobe represent great

candidates for use in brain activity mapping and related research. With further development and application, they can also extend substantially the scope of fundamental and applied electrophysiology studies and contribute to areas such as high-throughput drug screening.

2.4.3 *Metal Electrode-Based Intracellular Probes*

In addition to our active FET-based intracellular probes, several groups have been exploring the on-chip metal electrodes for intracellular action potential recording. The first example is the micrometer-sized protruding gold-spine microelectrode array [31, 32]. The mushroom-shaped Au electrodes here were functionalized with a peptide containing multiple Arg-Gly-Asp (RGD) repeats, named engulfment-promoting peptide (EPP9), to facilitate the engulfment of the gold-spine electrodes by the membrane of the cultured neurons thus forming a high-resistance seal. Following glass micropipette stimulation of nearby or the same cells, the engulfed gold-spine electrodes recorded subthreshold synaptic and action potential signals from individual neurons. From a representative measurement, the amplitude of the “raw” action potentials recorded by different spine electrodes was 0.1–25 mV, which is smaller than the value simultaneously recorded with a glass micropipette. Differences in the signal amplitude from different electrodes could arise from variations of the electrode impedance and/or the electrode/cell membrane coupling [31].

In another design, multiple vertical nanowires/nanopillars of metal, or silicon capped with metal, were used together as an electrode for intracellular recording. An example, named as vertical nanowire electrode arrays (VNEA) by the authors, uses a 3×3 array of vertical SiNWs (diameter of ~ 150 nm, length of ~ 3 μm) capped with a sputter-deposited metal tip for each intracellular probe [33]. Measurements from HEK293 and rat cortical neurons, which were cultured on the probes, suggested that some nanowire electrodes spontaneously penetrated HEK293 cell membranes during culture, although for others, a large voltage pulse ($\sim \pm 3$ V; duration, 100 ms) was needed to yield intracellular signals. The signals recorded with this SiNWs array electrode were $10\times$ smaller in amplitude than that simultaneously recorded with the glass patch pipette [33]. Another similar example uses multiple vertical platinum nanowires (PtNWs, diameter of ~ 150 nm, length of ~ 1.5 μm) for each intracellular probe, which is termed as nanopillar electrode. The direct recording using these nanopillar probes from HL-1 cells cultured on them initially yielded small ~ 100 – 200 μV extracellular spikes, but following voltage pulse (2.5 V; 200 μs), the signal transitions to one similar to an intracellular action potential albeit with a reduced amplitude of ~ 11.8 mV [34]. The authors suggest that the voltage pulse induces nanometer-sized pores thereby providing access to the intracellular potential. This electroporation mechanism was thought as an underlying mechanism for the electrical pulse-induced intracellular recording by the VNEA as well.

Compared to the nanoFET probes, the metal-based intracellular electrodes discussed here have an advantage in that the electrode can deliver stimulatory pulses to

evoke action potentials and act as a stimulator, in addition to potential detector. However, the dependence of the recording on electrode/electrolyte interface impedance limits the probe size to be in the micrometer scale (gold-spine electrode) or requires multiple nanowires per electrode (VNEA and nanopillar probes) which also results in overall size of electrodes in micrometer scale. In addition, nanoFET probes usually give larger signal amplitude and SNR than the metal-based electrodes. The overall comparison of different intracellular recording probes is summarized in Table 2.1 [35].

2.5 Merging Nanoelectronics with Artificial Tissues Seamlessly in Three Dimensions

Implementing electronic sensors in 3D and the capabilities for monitoring cells throughout the 3D microenvironment of tissues are critical for functional neural activity mapping and understanding physicochemical changes relevant to living organisms. Previous works have been limited in terms of merging electronics with tissues throughout 3D space with minimal tissue disruption, because the 2D support structures and the electronic sensors are generally on much larger scale than the extracellular matrix (ECM) and cells. The bottom-up paradigm from separately synthesized nanomaterials used for nanoFETs fabrication provides a unique opportunity to make freestanding, substrate-free nanoscale electrical probes that are distributed in 3D space. By merging these 3D probes with living tissues or artificial tissue constructs, electrical recording and mapping of cellular activities in 3D from inside the tissues could be realized. In this section, we will review the effort toward realizing the seamless merging between electronic system and artificial tissues through the fabrication of 3D macroporous, freestanding nanoscale devices.

2.5.1 *Three-Dimensional Macroporous Nanoelectronic Network*

Conceptually, the seamless merging between nanoelectronic network and artificial tissue in 3D can be achieved in three basic steps as follows (Fig. 2.15) [36, 37]: (1) fabrication of the nanoelectronic network/nanoprobe arrays in 2D with underlying sacrificial layers and substrate support (step A, Fig. 2.15); (2) removal of the sacrificial layers to release the nanoelectronic network to yield 3D, freestanding nanoelectronic scaffolds (nanoES), where the nanoES is used alone or combined with traditional tissue scaffold materials (step B, Fig. 2.15); and (3) cell seeding and culture on the nanoES to yield 3D nanoelectronic-tissue hybrids (step C, Fig. 2.15) [37]. In this new paradigm, the use of nanoES is the critical advance, which addresses key constraints as follows: (1) the electronic network must be

Table 2.1 Electrical and methodological specifications of the various modalities of intracellular electrical recordings:

	Electrode impedance (Ω)	Primary electrode material	Size	Seal (Ω)	Maximum AP (mV)	Maximum EPSP (mV)	Cell type studied	Coupling coefficient	Insertion method	Coating	Duration of stable recording	Ref.
Patch-clamp electrodes (whole cell)	10^6	Glass	micro-pipette	$>10^9$	^a	^a	Multiple	1	Mechanical	None	Hours	21
Kinked silicon nanowire FET	Irrelevant (FET device)	Si	Approximately several hundreds of nanometers	N/A	Full amplitude, 75–100	None	Cardiomyocytes	~1	Mechanical or spontaneous	Phospholipids	Hours	22
Branched intracellular nanotube FET	Irrelevant (FET device)	Si/SiO ₂	~30–150	N/A	Full amplitude, 75–100	None	Cardiomyocytes	~1	Mechanical or spontaneous	Phospholipids	Hours	26
Active silicon nanotube transistor	Irrelevant (FET device)	Si	~30–150	N/A	Full amplitude, 75–100	None	Cardiomyocytes	~1	Mechanical or spontaneous	Phospholipids	Hours	27
Gold mushroom-shaped MEA (g M μ E)	10^{11}	Au	1–2 μ m	10^6	25	5	Neurons (aplysia)	~0.5	Biological endocytosis	RGD motif peptide	>2 days	31
Vertical nanowire electrode arrays (VNEA)	3×10^8 (with application of bias volt- age = 1.5 V)	Si with Au tip	Multiple nanowires spread in a size of several micrometers	$>10^8$	4	None	None Neurons (cortical rat)	~0.3	Bias voltage	Silanization	10 min	33
Nanopillar electroporation	6×10^6 18×10^6 (depending on the number of pillars)	Pt	Multiple nanowires spread in a size of several μ m	N/A	12	None	HL-1 cell line	~0.1	Electroporating current	Fibronectin	10 min per daily session, 3 days	34
gM μ E electroporation	10^{11}	Au	1–2 μ m	10^8	6	None	Neurons (aplysia)	~0.1	Electroporating current	Poly-L-lysine	5 min	32

^aGlass micropipettes provide maximal (one-to-one) AP (action potential) and EPSP (excitatory postsynaptic potential) amplitudes

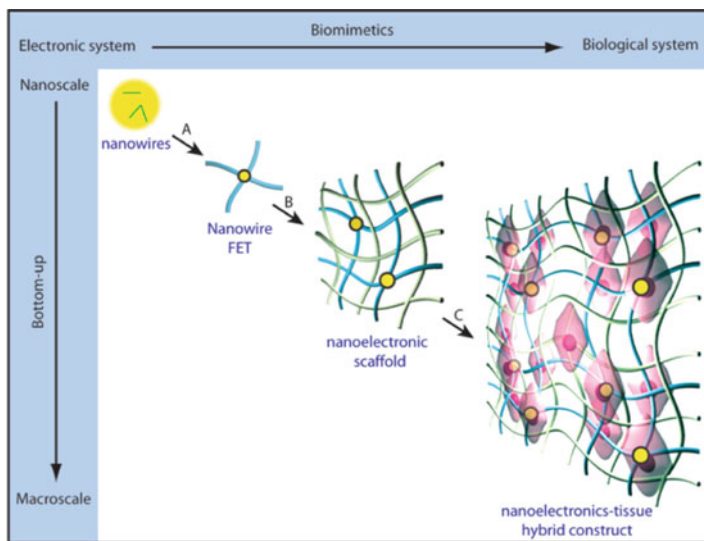


Fig. 2.15 Merging nanoelectronics with artificial tissues seamlessly in three dimensions [36]

macroporous (e.g., >99 % porosity), not planar, to enable 3D interpenetration with biomaterials and cells; (2) structural dimensions in nanometer to micrometer scale to match the natural ECM and mimic the well-studied scaffold materials used for tissue engineering and also to minimize the invasiveness; (3) 3D interconnectivity and addressability of the electronic devices; and (4) mechanical properties more compatible with the natural tissue (e.g., much softer than normal electronics).

Two basic types of nanoES have been developed and exploited for the creation of innervated tissues with neurons, cardiomyocytes, and smooth muscle cells. The first one, named reticular nanoES, uses stress in the bi- or tri-metallic interconnects to induce the self-bending and self-organization of the nanoelectronic network /nanoprobes array, in order to yield a 3D scaffold with interconnected and addressable nanowire FET sensors embedded in the different positions of the 3D space (Fig. 2.16a). Reconstructed 3D confocal fluorescence images of a typical reticular scaffold (Fig. 2.16b) shows clearly the 3D interconnected structure, with a magnified SEM image of one of the kinked SiNW FET sensor elements in the nanoES shown in Fig. 2.16c [36]. The second basic class of nanoES, the mesh nanoES, is prepared upon the use of a 2D macroporous nanoelectronic network sheet (Fig. 2.16d). A regular nanoelectronic devices array with structural backbone (e.g., made of Su-8) is patterned on the supporting substrate combining nanowire assembly and conventional 2D lithography (Fig. 2.16e). After release from the substrate with the removal of underlying sacrificial layer, the freestanding and flexible 2D macroporous nanoelectronic network sheet can be organized into 3D macroporous structures by either manual manipulation such as rolling (Fig. 2.16f) or stress-induced self-assembly [36, 37]. Usually, the manual manipulation gives more flexibility in making nanoES of large size than the stress-induced self-organization.

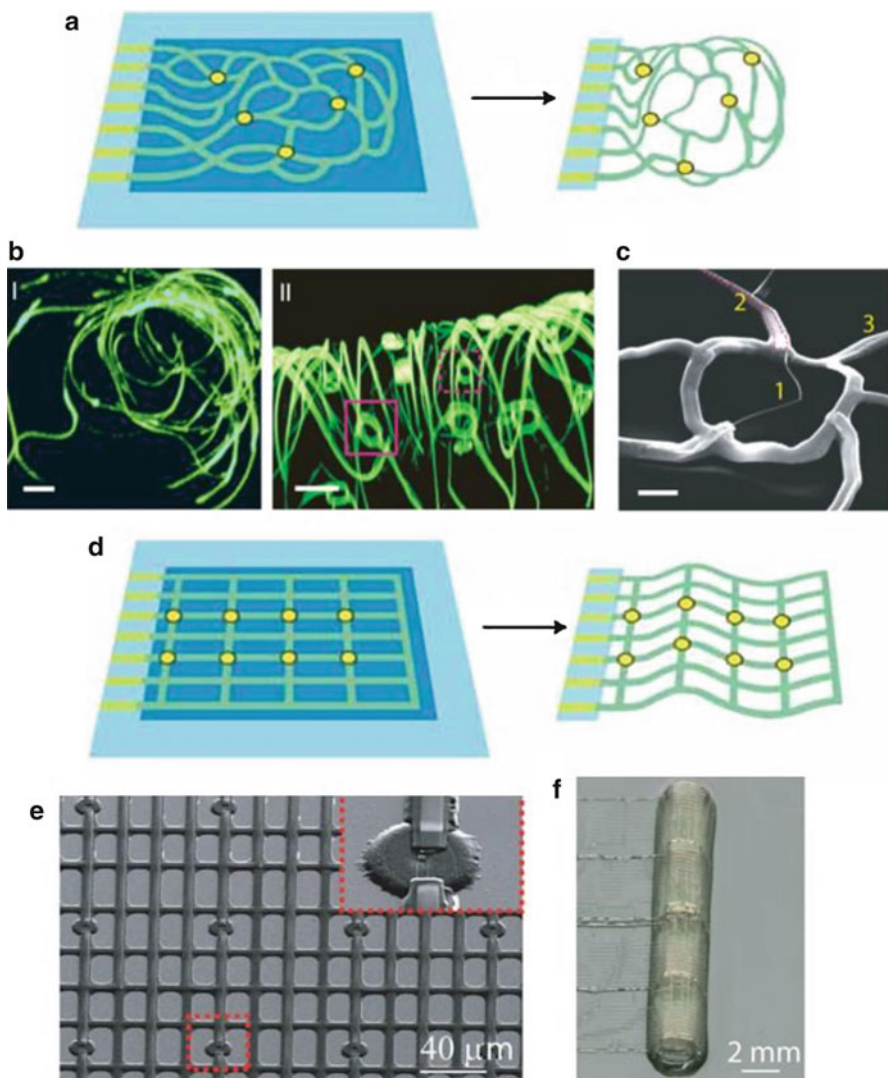


Fig. 2.16 Reticular and mesh nanoES: (a) device fabrication schematics for reticular nanoES. Light blue, silicon oxide substrates; blue, nickel sacrificial layers; green, nanoES; and yellow dots, individual nanowire FETs. (b) 3D reconstructed confocal fluorescence micrographs of a reticular nanoES viewed along the y (I) and x (II) axes. Solid and dashed open magenta squares indicate two SiNW FET devices located on different planes along the x axis. Scale bars, 20 μm . (c) SEM image of a single kinked SiNW FET within a reticular scaffold, showing (1) the kinked SiNW, (2) metallic interconnects (dashed magenta lines), and (3) the SU-8 backbone. Scale bar, 2 μm . (d) Device fabrication schematics for mesh nanoES. The color designation is same as (a). (e) SEM image of a 2D macroporous nanoelectronic network before release from the substrate. (Inset) Zoom-in of the region enclosed by the small red dashed box containing a single SiNW FET device [37]. (f) Photograph of a manually scrolled-up 3D macroporous mesh nanoES [37]. (a–d) [36]

In the nanoES, the 3D networks can have porosities larger than 99 %, ready for 3D interpenetration with cells. It can also contain hundreds of addressable nanowire devices and have feature sizes from the 1–10 μm scale (for electrical and structural interconnections) to the 10 nm scale (for device elements) [36, 37]. This size represents minimal invasiveness to the tissues, as reflected by the cytotoxicity tests which showed minimal difference in cell viability for culture in the scaffold with and without nanoES. Importantly, typical 3D macroporous nanoelectronic networks are reported to have a very low effective bending stiffness from 0.0038 to 0.0378 nN/m [37]. These values, which can be readily tuned by changing the materials and size of the nanoES components, are comparable with synthetic and natural ECMs, thus making them an ideal scaffold for innervating synthetic neural and cardiac tissue.

2.5.2 *Three-Dimensional Nanoelectronics/Tissue Hybrids*

Before cell seeding and culture, the reticular and mesh nanoES are always merged with conventional macroporous biomaterials. Specifically, gel casting, lyophilization, and electrospinning were used to deposit and construct macroporous collagen, alginate, and poly(lactic-co-glycolic acid) (PLGA), respectively, around nanoES. Embryonic rat hippocampal neurons were cultured on the reticular nanoES/Matrigel scaffold [36]. Reconstructed 3D confocal micrographs from a 2-week culture (Fig. 2.17a, b) show clearly neurons with a high density of spatially interconnected neurites that penetrate seamlessly the reticular nanoES (Fig. 2.17a), sometimes passing through the ring structures supporting individual SiNW FET sensors (Fig. 2.17b) [36]. Nanoelectronics/cardiac tissue was constructed from a hybrid mesh nanoES/PLGA scaffold following seeding and culture of cardiomyocytes. Confocal fluorescence microscopy of a typical cardiac 3D culture (Fig. 2.17c) revealed a high density of cardiomyocytes in close contact with the nanoES components. Epifluorescence micrographs of cardiac cells on the surface (Fig. 2.17d) further show striations characteristic of cardiac tissue [36]. Significantly, the original SiNW FET device characteristics were retained after the nanoES 3D organization, scaffold hybridization, and cell culture up to at least 12 weeks. This capability of the nanoES for long-term culture and monitoring is important for 3D recording and mapping of neural and cardiac activities. It also enables a number of in vitro studies, including drug screening assays with these synthetic neural and cardiac tissues, where 3D tissue models will provide a more robust link to in vivo disease treatment than the 2D cell cultures.

The recording of extracellular action potentials by the SiNW FET devices within the nanoES/cardiac tissue hybrid demonstrated the 3D monitoring capabilities of the nanoES. As shown in Fig. 2.17e, a SiNW FET $\sim 200\ \mu\text{m}$ below the construct surface gave regularly spaced spikes with a frequency of $\sim 1\ \text{Hz}$, a calibrated potential change of $\sim 2\text{--}3\ \text{mV}$, a $\text{SNR} \geq 3$, and a $\sim 2\ \text{ms}$ width, which is consistent with the standard extracellular signals from cardiomyocytes. Following dosage of the construct with noradrenaline (also known as norepinephrine), which is a drug that

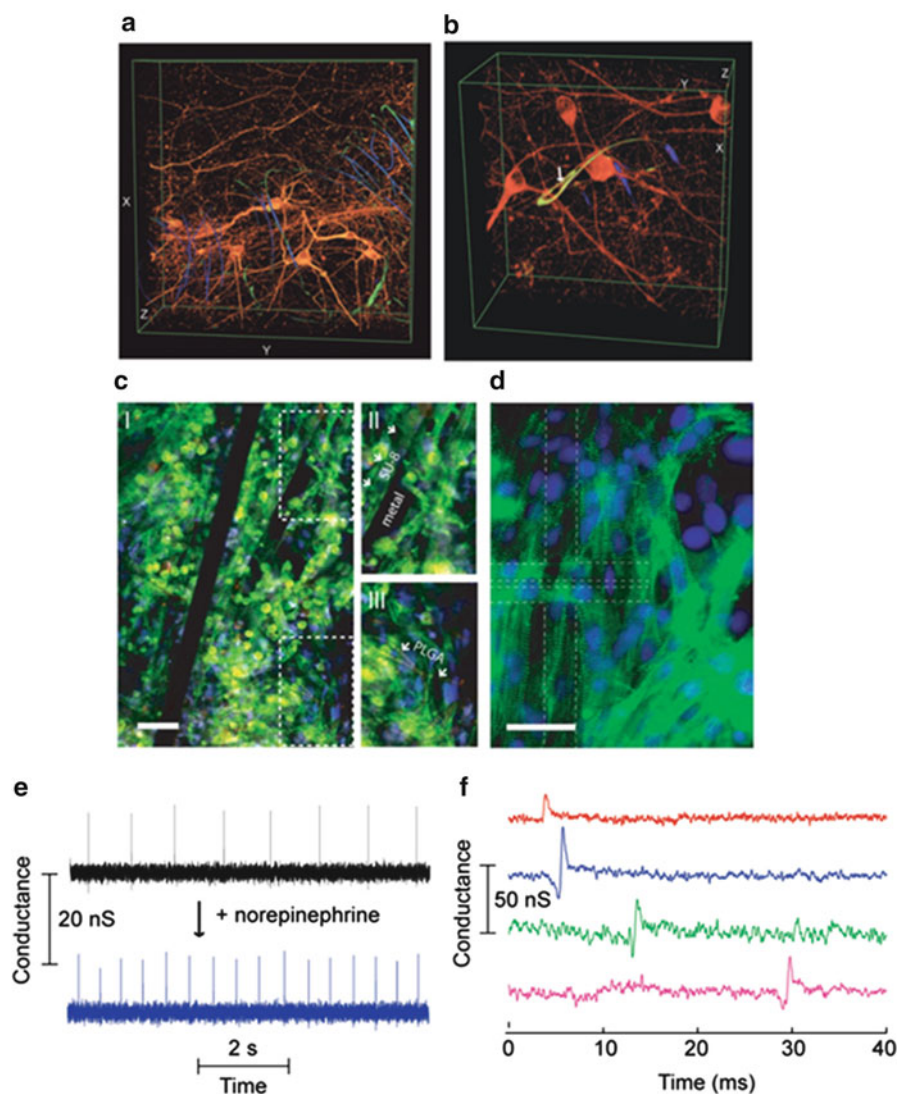


Fig. 2.17 3D nanoelectronics/tissue hybrids: (a, b) 3D reconstructed confocal images of rat hippocampal neurons after a 2-week culture on a reticular nanoES/Matrigel scaffold. The white arrow highlights a neurite passing through a ringlike structure supporting a nanowire FET. Dimensions in (a) x, 317 μm ; y, 317 μm ; and z, 100 μm and in (b) x, 127 μm ; y, 127 μm ; and z, 68 μm . (c) Confocal fluorescence micrographs of a synthetic cardiac patch. (II and III), Zoomed-in view of the upper and lower dashed regions in I, scale bar, 40 μm . (d) Epifluorescence micrograph of the surface of the cardiac patch. Green, α -actin; blue, cell nuclei. The dashed lines outline the position of the S/D electrodes. Scale bar, 40 μm . (e) Conductance versus time traces recorded from a single SiNW FET before (black) and after (blue) applying noradrenaline. (f) Multiplexed electrical recording of extracellular action potentials from four SiNW FETs at different depths in a nanoES/cardiac hybrid [36]

stimulates cardiac contraction, the recorded signal showed a twofold increase in action potential firing frequency [36]. It was reported that SiNW FETs at different position in the cardiac patch showed response to the noradrenaline application at different time scale. In addition, multiplexed recording from a coherently beating nanoES/cardiac construct demonstrated submillisecond temporal resolution from the four SiNW FETs with separations up to 6.8 mm within the 3D innervated cardiac tissue sample (Fig. 2.17f) [36].

The studies on the nanoelectronics/tissue hybrid and 3D neural and cardiac activity mapping are still at their infancy. However, the concept and strategy of making 3D macroporous nanoES discussed here provides a promising way toward this direction. The bottom-up paradigm started from separately synthesized nanomaterials to make the nanoprobe is the key for the 3D, freestanding nanoES. And the small size of these nanoprobe represents minimal invasiveness, which is critical for long-term and stable 3D recording. Further development of the nanoES could not only have a profound impact on areas such as 3D brain activity mapping and in vitro pharmacological studies but also suggest possibilities for implantable “cyborg” tissues enabling closed-loop monitoring and treatment of diseases.

2.6 Summary

This chapter reviews comprehensively the recent development of novel nanoscale probes, mainly FET-based nanoprobe, as extra- and intracellular action potential recording probes for biological systems ranging from single cultured cells to tissue slices and whole organs, through 3D artificial tissue constructs. The small size of these nanoscale probes minimizes the invasiveness and also increases the spatial resolution for the recording. The enabled large area, high-density extra- and intracellular recording of action potentials with cellular and subcellular resolution will be important for neural and cardiac activity mapping. Furthermore, the bottom-up paradigm used to fabricate these nanodevices allows for the development of 3D, freestanding nanoprobe which adds the capability for recording throughout the 3D space inside the artificial tissue constructs and opens up exciting possibilities for 3D neural activity mapping. All of these results represent great advances in nanoelectronics-biology interfacing and neural activity recording and could serve as foundations for many new fundamental studies and novel biomedical applications in neuroscience [38, 39].

References

1. Sze, S.M., Ng, K.K.: *Physics of Semiconductor Devices*, 3rd ed., Wiley Interscience, New York (2006)
2. Patolsky, F., Zheng, G., Lieber, C.M.: Nanowire-based biosensors. *Anal. Chem.* **78**, 4260–4269 (2006)

3. Timko, B.P., Cohen-Karni, T., Qing, Q., Tian, B., Lieber, C.M.: Design and implementation of functional nanoelectronic interfaces with biomolecules, cells, and tissue using nanowire device arrays. *IEEE Trans. Nanotechnol.* **9**, 269–280 (2010)
4. Lieber, C.M.: Nanoscale science and technology: building a big future from small things. *MRS Bull.* **28**, 486–491 (2003)
5. Wagner, R. S.: *Whisker Technology*, Wiley, New York (1970)
6. Lu, W., Lieber, C.M.: Semiconductor nanowires. *J. Phys. D.: Appl. Phys.* **39**, R387–R406 (2006)
7. Gudiksen, M.S., Lauhon, L.J., Wang, J., Smith, D.C., Lieber, C.M.: Growth of nanowire superlattice structures for nanoscale photonics and electronics. *Nature* **415**, 617–620 (2002)
8. Lauhon, L.J., Gudiksen, M.S., Wang, C.L., Lieber, C.M.: Epitaxial core–shell and core–multishell nanowire heterostructures. *Nature* **420**, 57–61 (2002)
9. Jiang, X., Tian, B., Xiang, J., Qian, F., Zheng, G., Wang, H., et al.: Rational growth of branched nanowire heterostructures with synthetically encoded properties and function. *Proc. Natl. Acad. Sci. U S A* **108**, 12212–12216 (2011)
10. Tian, B., Xie, P., Kempa, T.J., Bell, D.C., Lieber, C.M.: Single-crystalline kinked semiconductor nanowire superstructures. *Nature Nanotech.* **4**, 824–829 (2009)
11. Erickson, J., Tooker, A., Tai, Y.-C., Pine, J.: Caged neuron MEA: A system for long-term investigation of cultured neural network connectivity. *J. Neurosci. Method.* **175**, 1–16 (2008)
12. Patolsky, F., Timko, B.P., Yu, G., Fang, Y., Greytak, A.B., Zheng, G., et al.: Detection, stimulation, and inhibition of neuronal signals with high-density nanowire transistor arrays. *Science* **313**, 1100–1104 (2006)
13. Cohen-Karni, T., Timko, B.P., Weiss, L.E., Lieber, C.M.: Flexible electrical recording from cells using nanowire transistor arrays. *Proc. Natl. Acad. Sci. U S A* **106**, 7309–7313 (2009)
14. Cohen-Karni, T., Qing, Q., Li, Q., Fang, Y., Lieber, C.M.: Graphene and nanowire transistors for cellular interfaces and electrical recording. *Nano Lett.* **10**, 1098–1102 (2010)
15. Hu, Y., Xiang, J., Liang, G., Yan, H., Lieber, C.M.: Sub-100 nanometer channel length Ge/Si nanowire transistors with potential for 2 THz switching speed. *Nano Lett.* **8**, 925–930 (2008)
16. Cohen-Karni, T., Casanova, D., Cahoon, J., Qing, Q., Bell, D., Lieber, C.M.: Synthetically encoded ultrashort-channel nanowire transistors for fast, pointlike cellular signal detection. *Nano Lett.* **12**, 2639–2644 (2012)
17. Kodambaka, S., Tersoff, J., Reuter, M.C., Ross, F.M.: Germanium nanowire growth below the eutectic temperature. *Science* **316**, 729–732 (2007)
18. Hille, B.: *Ion Channels of Excitable Membranes*, 3rd ed., Sinauer Associates Inc., Sunderland (2001)
19. Qing, Q., Pal, S.K., Tian, B., Duan, X., Timko, B.P., Cohen-Karni, T., et al.: Nanowire transistor arrays for mapping neural circuits in acute brain slices. *Proc. Natl. Acad. Sci. U S A* **107**, 1882–1887 (2010)
20. Timko, B.P., Cohen-Karni, T., Yu, G., Qing, Q., Tian, B., Lieber, C. M.: Electrical recording from hearts with flexible nanowire device arrays. *Nano Lett.* **9**, 914–918 (2009)
21. Molleman, A.: *Patch Clamping: An Introductory Guide to Patch Clamp Electrophysiology*. John Wiley & Sons, Ltd., Chichester, West Sussex, England (2003)
22. Tian, B., Cohen-Karni, T., Qing, Q., Duan, X., Xie, P., Lieber, C.M.: Three-dimensional, flexible nanoscale field-effect transistors as localized bioprobes. *Science* **329**, 830–834 (2010)
23. Jiang, Z., Qing, Q., Xie, P., Gao, R., Lieber, C.M.: Kinked p-n junction nanowire probes for high spatial resolution sensing and intracellular recording. *Nano Lett.* **12**, 1711–1716 (2012)
24. Weber, L., Gmelin, E.: Transport properties of silicon *Appl. Phys. A* **53**, 136–140 (1991)
25. Xu, L., Jiang, Z., Qing, Q., Mai, L., Zhang, Q., Lieber, C.M.: Design and synthesis of diverse functional kinked nanowire structures for nanoelectronic bioprobes. *Nano Lett.*, **13**, 746–751 (2013)
26. Duan, X., Gao, R., Xie, P., Cohen-Karni, T., Qing, Q., Choe, H.S., et al.: Intracellular recordings of action potentials by an extracellular nanoscale field-effect transistor. *Nature Nanotech.* **7**, 174–179 (2012)
27. Gao, R., Strehle, S., Tian, B., Cohen-Karni, T., Xie, P., Duan, X., et al.: Outside looking in: nanotube transistor intracellular sensors, *Nano Lett.* **12**, 3329–3333 (2012)

28. Goldberger, J., Hochbaum, A.I., Fan, R., Yang, P.: Silicon vertically integrated nanowire field effect transistors. *Nano Lett.* **6**, 973–977 (2006)
29. Almquist, B.D., Melosh, N.A.: Fusion of biomimetic stealth probes into lipid bilayer cores. *Proc. Natl. Acad. Sci. U S A*. **107**, 5815–5820 (2010)
30. Alivisatos, P.A., Andrews, A.M., Boyden, E.S., et al.: Nanotools for neuroscience and brain activity mapping. *ACS Nano* **7**, 1850–1866 (2013)
31. Hai, A., Shappir, J., Spira, M.E.: In-cell recordings by extracellular microelectrodes. *Nature Methods* **7**, 200–202 (2010)
32. Hai, A., Spira, M.E.: On-chip electroporation, membrane repair dynamics and transient in-cell recordings by arrays of gold mushroom-shaped microelectrodes. *Lab Chip* **12**, 2865–2873 (2012)
33. Robinson, J.T., Jorgolli, M., Shalek, A.K., Yoon, M-H., Gertner, R.S., Park, H.: Vertical nanowire electrode arrays as a scalable platform for intracellular interfacing to neuronal circuits. *Nature Nanotech.* **7**, 180–184 (2012)
34. Xie, C., Lin, Z., Hanson, L., Cui, Y., Cui, B.: Intracellular recording of action potentials by nanopillar electroporation. *Nature Nanotech.* **7**, 185–190 (2012)
35. Spira, M.E., Hai, A.: Multi-electrode array technologies for neuroscience and cardiology. *Nature Nanotech.* **8**, 83–94 (2013)
36. Tian, B., Liu, J., Dvir, T., Jin, L., Tsui, J.H., Qing, Q., et al.: Macroporous nanowire nanoelectronic scaffolds for synthetic tissues. *Nature Mater.* **11**, 986–994 (2012)
37. Liu, J., Xie, C., Dai, X., Jin, L., Zhou, W., Lieber, C.M.: Multifunctional three-dimensional macroporous nanoelectronic networks for smart materials. *Proc. Natl. Acad. Sci. U S A* **110**, 6694–6699 (2013)
38. Duan, X., Lieber, C.M.: Nanoelectronics meets biology: from new nanoscale devices for live-cell recording to 3D innervated tissues. *Chem.-Asian J.* **8**, 2304–2314 (2013)
39. Duan, X., Fu, T.-M., Liu, J., Lieber, C.M.: Nanoelectronics-biology frontier: from nanoscopic probes for action potential recording in live cells to three-dimensional cyborg tissues. *Nano Today* **8**, 351–373 (2013)

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