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Synthetic Nanoelectronic Probes for Biological Cells and Tissue

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Abstract

Research at the interface between nanoscience and biology has the potential to produce breakthroughs in fundamental science and lead to revolutionary technologies. In this review, we focus on nanoelectronic/biological interfaces. First, we discuss nanoscale field effect transistors (nanoFETs) as probes to study cellular systems, including the realization of nanoFET comparable in size to biological nanostructures involved in communication using synthesized nanowires. Second, we overview current progress in multiplexed extracellular sensing using planar nanoFET arrays. Third, we describe the design and implementation of three distinct nanoFETs used to realize the first intracellular electrical recording from single cells. Fourth, we present recent progress in merging electronic and biological systems at the 3D tissue level by using macroporous nanoelectronic scaffolds. Finally, we discuss future development in this research area, the unique challenges and opportunities, and the tremendous impact these nanoFET based technologies might have in advancing biology and medical sciences.

Keywords

Nanowire; field effect transistor; intracellular; extracellular; synthetic tissue

1. INTRODUCTION

Semiconductor science and technology is a driving force of the modern society due to the ever-increasing miniaturization of semiconductor processing and transistor devices(1–6). To continue the remarkable success of semiconductor technology and possibly produce new paradigms for logic, memory and sensor devices, many researchers have been investigating devices based on synthesized nanostructures(2,5,7–12) in which geometries, organizations and physical properties can be designed and controlled at the nanometer scale.

A wide spectrum of nanostructured materials have been designed and synthesized over the past several decades, including colloidal nanoparticles(13,14), semiconductor nanowires (NW)(3,4,15,16), and graphene(10,17–20), where properties distinct from their bulk counterparts have been discovered and exploited. For any class of nanostructured materials to become a platform for discovery and development, it is critical that new structures and

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assemblies with tunable composition, morphology, and properties at different length scales be obtainable(3,10,18). In this regard, semiconductor nanowires have been recognized as one of the most successful platforms available today in nanoscience. First, it is now possible to design nanowire structures *de novo* and synthetically realize these structures with complex, yet controlled, modulations in composition(8,16,21–26), doping(16,23), defect(27–29) and even topography(30–32). Second, this high-level of synthetic control enables nanowire building blocks to be created that have predictable physical properties for testing fundamental limits of performance(5,16). Third, it is now possible to assemble hybrid or multicomponent functional materials in novel layout and configuration using these diverse nanowire building blocks(31,33–45), allowing for rational exploration of the possible applications of multi-component materials. With these characteristics and capabilities, nanowires are ideal building blocks for exploring what is possible in nanoscience and also creating new technologies. This has been the focus in nanoscience community over the past decade and continues to be so as it crosses over other disciplines, such as synthetic biology(46–51).

Research at the interface between nanoscience and biology has the potential to produce breakthroughs in fundamental sciences and lead to revolutionary technologies(52,53). In particular, the exploration and application of semiconductor nanowire materials and devices in cellular systems could produce unprecedented interactions down to the molecular level. Such interactions have been utilized to gain insights especially those relevant to human health by stimulating, recording from and delivering objects to single cells and tissues in controlled ways to induce desired physiological responses, while minimizing undesirable effects(52,53).

There are two types of nanowire-based platforms in biomedical sciences: *basic platforms* that can be readily adapted to address biomedical questions; and *advanced platforms* that are specifically designed to push the frontiers of what is possible by, for example, enabling a new measurement tool. The *basic platforms* use conventional nanowire material and device systems with well-exploited physical or chemical properties, and they also have wide-ranging applications in many other fields, such as energy scavenging systems(54–61) or components for integrated circuit(34,35). These *basic platforms*, such as planar nanowire field effect transistors(34,35,37,40,43) or vertical nanowire arrays(55–58,60,61), have been used in biomolecular sensing(52,53), extracellular recording(52,53), drug delivery(62–64) and localized cellular imaging(65). On the other side, the *advanced platforms* have been designed to address some intrinsic complexity in biology and medical sciences in way simply not possible previously. They allow new types or new scales of interact and measurements with their target systems(31,66–68), and in so doing, open up completely new opportunities in science and technology. Examples of *advanced platforms* include recent intracellular field effect transistor probes(31,67–69) and nanoelectronics-innervated synthetic tissues(66).

This review discusses the basic concepts of nanoscale field effect transistors (nanoFETs) and their applications in cellular electrophysiology. The first section highlights the motivation behind nanoFET probes to study cellular systems versus existing recording technologies, followed by the introduction of chemical synthesis to realize nanoFETs *de novo*. The second section gives an overview of the current progress in multiplexed extracellular sensing using planar nanoFET arrays. Electrical recordings at single cell, tissue and organ levels will be discussed, and their limits and promises will be delineated. The third section will detail the main designs and implementations of nanoFETs in intracellular electrical recording from single cells, the first paradigm change in intracellular electrophysiology since the 1950s. NanoFET based techniques will be compared with conventional micropipette and microelectrode probes, and the limits and future opportunities

of these new probes will be discussed. The fourth section will introduce very recent progress in merging electronic and biological systems at the 3D tissue level by introducing the new concept of macroporous nanoelectronic scaffolds. The first-ever nanoelectronics 'innervated' synthetic tissues will be reviewed and their applications will be discussed. The final section will present our perspectives on future development in this research area, the unique challenges and opportunities, and the tremendous impact these nanoFET based technologies might have in advancing biology and medical sciences.

2. FUNDAMENTALS OF NANOFET

2.1. Why and how are nanoFETs applied in biology and medicine?

The ability to make electrical measurements inside single cells or throughout the entire 3D space of the tissue can have many important impacts in electrophysiology and biomedical sciences. The patch clamp technique, in which a pulled glass micropipette filled with electrolyte is inserted into a cell, offers intracellular electrical measurements with high signal-to-noise ratio (S/N) and single ion channel recording capability(70). Ideally, the micropipette should be as small as possible to increase the spatial resolution and reduce the invasiveness of the measurement, and ideally, allow for recording from subcellular structures. However, the overall performance of the technique also depends on the impedance of the interface between the micropipette and the cell interior (*i.e.*, the smaller the probe tip size, the larger the junction impedance), which sets limits on the temporal resolution and S/N of the micropipette-based electrical probes(31,41). Advanced techniques that involve inserting metal or carbon microelectrodes or nanoelectrodes into cells or tissues could be subject to similar dilemma, because all these tools are single terminal devices and electrochemical thermodynamics and kinetics must be considered for device operation(71–78). We will discuss them in details in the subsequent sections.

In integrated circuits, the basic device element is a multi-terminal FET that uses either electrons or holes as the charge carriers(79) (Figure 1a). Although the charge carriers are ions in biological systems, there are many biophysical links that connect ions to electrons and holes in a FET. For example, the dynamic flow of ions in biological system can generate spatially defined field potential(80). The Poisson equation(81) links such potentials directly to the ionic current sources and sinks that produce them. The Goldman-Hodgkin-Katz voltage equation(81) has also been used in cell membrane physiology to determine the equilibrium potential across a cell's membrane, where it takes into account all of the ions that permeate through that membrane. The potentials, generated by ion flows and gradients, can function as the gate signals to modulate the electrical output in FET devices (Figure 1b and 1c). The sensitivity of a FET or how well the transistor can receive and amplify the gate signal is usually defined as transconductance (G_m)(6,52,53,79), which is inversely proportional to the dimension (L) of the active device(6). This fact implies that the use of nanoelectronics would have improved sensitivity compared to its bulk and planar counterparts. As shown in the following sections, nanoFETs have shown to be able to record electric potentials inside cells(31,67–69) and from the internal regions of synthetic tissues(66), and because their performance does not depend on impedance, they can be made much smaller than micropipettes and microelectrodes. Moreover, nanoFET arrays are better suited for multiplexed measurements(67,68).

2.2. Chemical synthesis of nanoFETs

Three distinct classes of *de novo* design and synthesis have been used to yield nanoFETs building blocks , covering structural motifs in one-dimension (1D), 2D and 3D (Figure 2). The basic semiconductor nanowire structure (Figure 2a, I) consists of a uniform composition, 1D structure with a diameter typically in the range of 3–500 nm. In the growth

process, which builds upon earlier work showing vapor-liquid-solid (VLS) growth of micrometer to millimeter diameter wires(82,83), the nanocluster catalyst (typically gold nanoparticles) forms a liquid solution with nanowire reactant component(s), and when supersaturated, acts as the nucleation site for crystallization and preferential 1D growth(84,85). Other growth mechanisms, such as vapor-solid-solid (VSS) and vapor-solid (VS)(15), can also be explored to yield high quality semiconductor nanowires. Within this framework, it is straightforward to synthesize nanowires with different compositions, such as groups III-V, IV and II-VI semiconductors(8,15,86,87), using the appropriate nanocluster catalysts and growth temperatures/pressures. Additionally, nanowire structures in which the composition, dopant and even growth mechanisms (*e.g.*, VLS, VSS) are modulated along axial(21,22,88–90) (Figure 2b) or radial directions(25,29,91)have also been widely exploited. These axial and radial nanowire heterostructures provide a number of advantages compared to homogeneous semiconductor nanowires, and they have proven exceptionally powerful for a broad range of electronic, photonic and optoelectronic device applications(16). For example, germanium/silicon core/shell nanowires have been chemically synthesized for high mobility nanowire FETs due to quantum confinement of carriers within the germanium core by the larger band-gap silicon shell(5,92–95).

The second structural motif was recently demonstrated by an approach in which topological centers are synthetically introduced in a controlled manner in linear 1D structures (Figure 2a, II)(31,32). In this area, we demonstrated that iterative control over nucleation and growth leads to kinked nanowires, in which the straight sections are separated by triangular joints and where doping can be varied at these topologically defined points (Figure 2c). Moreover, new work has shown that it is possible to control the stereochemistry of adjacent kinks in a manner that allows the synthesis of increasingly complex two- and three-dimensional structures akin to organic chemistry, thus opening up a great opportunity for the future in terms of designed synthesis(31).

A third basic motif involves the synthesis of branched or tree-like nanowire structures (Figure 2a, III)(24,26,96). To this end, we reported a rational, multistep approach toward the general synthesis of 3D branched nanowire heterostructures(24). Single-crystalline semiconductor, including groups IV, III–V, and II–VI, and metal branches have been selectively grown on core or core/shell nanowire backbones, with the composition, morphology, and doping of core (core/shell) nanowires and branch nanowireswell controlled during synthesis.

Although the first structural motif has been used most extensively as building blocks of *basic platforms*, the second and third motifs have much higher level of structural and functional complexity, and show great potential of bottom-up synthesis to yield increasingly powerful functional building blocks for *advanced platforms*.

3. MULTIPLEXED EXTRACELLULAR ELECTRICAL RECORDING

3.1. Why nanoFETs for multiplexed extracellular recording

Natural and synthetic cellular assemblies are usually organized into 2D or 3D hierarchical networks operating on spatial and temporal scales that span multiple orders of magnitude. Advances in microfabrication of high-density passive multielectrode arrays (MEAs) and active transistor arrays on silicon substrates enable direct electrical recording down to ca. 10 micrometer length scales, although it is important to recognize that signals recorded within ~100 μm are often correlated[4–6], and moreover, it has been difficult to to resolve the cellular signals at the single cell level. As mentioned above, simply reducing the size of individual metal electrodes to achieve more localized detection is not viable due to corresponding

increases in their impedance[7,8], which intrinsically limits the resolution of such passive recording devices.

Silicon nanowire nanoFET arrays have several features that make them unique for high-resolution multiplexed extracellular recording from cellular systems. First, previous studies have shown that nanowire nanoFETs can exhibit ultra-high sensitivity detection of charged biomolecules, including detection of single particles(53). Second, bottom-up fabrication of nanoFETs yields devices that have nanoscale protrusions from the substrate surface(53,97). This can reduce device to cell/tissue separation and promote enhanced cell-nanostructure interaction and has resulted in high S/N extracellular recording of field potentials from cultured cells and cardiac tissue with signals improved compared to planar FETs. Third, the bottom-up approach also enables high-performance nanoFET fabrication on transparent, flexible and stretchable substrates(34,38–40). The freedom to design device structures and arrays on substrates adapted to specific biological applications also opens up new possibilities for interfacing with living tissues, for example, bio-resorbable and implantable devices(98–101). This freedom also allows other measurements or manipulations to be performed in conjunction with nanoFET recordings, such as high-resolution optical imaging. Fourth, the active junction area of typical nanoFETs, 0.01~ 0.1μm^2, is much smaller and can provide better spatial resolution of signals compared to MEA and planar FETs that are 10^2 to 10^5 times larger in active area(41). Last, nanoFET detectors provide fast intrinsic response time which is critical for high temporal resolution recordings(95,102).

3.2. Electrical interfacing with cultured neurons

An early example of multiplexed nanoFET recording layout consists of a neonatal rat cortical neuron and four peripheral silicon nanoFETs that are arranged at the corners of a rectangle, where polylysine patterning was used to promote axon and dendrites growth across single nanoFETs(103) (Figure 3a). This multiplexed nanoFET/neurite hybrid was used to study spike propagation with NW1 as a local input to elicit action potential spikes. After stimulation with a biphasic pulse sequence, back propagation of the elicited action potential was detected in the two dendrites crossing elements NW2 and NW3. The lack of observed signal from NW4 demonstrates the absence of crosstalk in the hybrid device array, and thus the capability for multiplexed subcellular resolution detection.

3.3. Recording from cardiomyocyte monolayers

We also carried out multiplexed measurements using the nanoFET arrays interfaced with cultured embryonic chicken cardiomyocytes (Figure 3b)(33). The nanoFETs were patterned in a linear array with an average spacing of 300 μm so that signal propagation within cardiomyocyte monolayers could be characterized. Recording from multiple nanoFETs in contact with spontaneously beating monolayer yielded very stable and high S/N (>10) field potential spikes. In this experiment, the relative large signal magnitude confirmed that a good junction is formed between each of the nanoFETs and PDMS/cell substrate. Additionally, a cross-correlation method was used to determine robustly the time differences between the signals recorded by the devices. The time shifts between devices and device separations yielded propagation speeds of 0.07–0.21 m/s that are consistent with other measurement on cardiomyocyte monolayers. The variation in propagation speeds in these initial studies is not surprising given the monolayer inhomogeneity and suggests an important future direction. We suggest that high-resolution multiplexed nanoFET recording together with optical imaging will enable details of intercellular propagation to be characterized for well-defined cellular structures.

3.4. Recording from tissues and organs

Finally, nanoFETs have been used to probe electrical activities from tissues and organs(41,42). To this end, we have studied the activity patterns of layer II/III cells in the piriform cortex of acute rat brain slices by stimulating different sets of axon fibers in the lateral olfactory tract (LOT). In a representative experiment, eight devices within a four-by-four 2D array oriented under the pyramidal cell layer of an acute slice were simultaneously monitored following stimulation at eight different spots (*a–h*) in the LOT(41) (Figure 3c). Strong stimulation of all axons fibers in the LOT yielded similar response by nanoFETs *1–8* with clear population spike signals (postsynaptic activities) regardless of stimulation positions. Reduced stimulation intensity was also used so that at each spot only a subgroup of fibers was activated. Notably, visual inspection of 2D activity maps for each of the eight stimulation positions demonstrates clearly how heterogeneous activity can be resolved (Figure 3d), and thus define a complex functional connectivity in the piriform cortex.

3.5. Challenges and promises

Although great progress has been made in the extracellular electrical recordings using nanowire nanoFETs, many challenges remain. For example, there is still a pressing need to further enhance the nanoFET S/N so that very weak endogenous biological signals, with the amplitude of ~ 100 μV, can be readily resolved. We can potentially achieve this goal by (1) new chemical design and synthesis of high mobility nanowire building blocks for nanoFET, (2) nanoscale engineering of nanowire materials to reduce nanoFET noise by, for example, thermal annealing and/or surface passivation.

It is also important to note that the high input impedance of the nanoFETs circumvents the common challenges confronted by implanted microelectrodes, where gradual increases of single terminal device impedance due to, for example, absorption of proteins, leads to degraded S/N over time(41,104,105). This feature makes nanoFETs very promising for multiplexed, in vivo chronic recordings. This is particularly true considering the facts that (i) nanoscale device feature size allows integration of multiple nanoFETs on minimally invasive and movable electrophysiological probes(68), (ii) bottom-up fabrication makes it possible to choose biocompatible or even biodegradable materials as substrates to reduce mechanical mismatch and to minimize inflammatory tissue response(31,66,68,98–101), and (iii) the nanoscale topology could be arbitrarily designed *de novo* to promote better attachment of single cells or even intracellular contacts. Therefore, nanoFETs should bring many exciting opportunities to interfacing living tissue and organs with electronics for biomedical applications (*e.g.*, diagnostic devices for brain trauma and surgical tools for cardiac therapy), and even new cybernetic biosystems for hybrid information processing.

4. INTRACELLULAR ELECTRICAL RECORDING

4.1. Why intracellular?

As the key cellular component, lipid membranes represent important structural and protective elements of the cell that form a stable, self-healing, and virtually impenetrable barrier to the ions and small molecules(106). Since these membranes have resistance (R) and capacitance (C), the membrane RC circuit also behaves as an electrical barrier and would attenuate and even distort the intracellular signals as they are detected by extracellular sensors. More importantly, although cellular signal transduction often starts with an extracellular signaling molecules activating a cell surface receptor, it is the subsequent intracellular processing that eventually creates a cellular response. Deciphering of such intracellular signal transmission and amplification processes is critical to the understanding of cellular information flow and cell physiology. Therefore, it is highly desirable to deliver

nanoFETs into the cell and directly record intracellular electrical activities, which can provide much more detailed understanding of the inner workings of cells..

4.2. Why nanoFETs for intracellular recording?

Although nanoFETs have been exploited for ultrasensitive detection of biological markers and high-resolution extracellular recording from cells(53), localized and tunable intracellular sensing and recording had not been demonstrated prior to our work because all FET and nanoFET devices were created on planar substrates --- using the *basic nanoFET platform*. Ideally, rather than force the cell to conform to the substrate, a movable and 3D nanoFET with the necessary source (S) and drain (D) electrical connections could move into contact with the cell and probe within the cell membrane. However, minimally invasive insertion of a nanoFET into the confined 3D space of single cells, or even 3D cellular networks, was still a major challenge before year 2010 because the S and D typically dominated the overall device size and defined a planar and rigid structure, regardless of whether the nanoFET was on or suspended above a substrate. An *advanced nanoFET platform* that is designed specifically for intracellular measurement is needed to meet this requirement(32,67–69). Three distinct examples that we have recently introduced to address this central challenge are shown schematically in Figure 4a, and include (1) kinked nanowire nanoFET, (2) branched-intracellular nanotube nanoFET, and (3) active nanotube nanoFET devices.

Existing probes capable of intracellular sensing and recording include voltage-sensitive optical dyes or proteins(107–110), and single-terminal glass or carbon microelectrodes as mentioned briefly in prior section(70,72) (Figure 5). Voltage-sensitive dyes can readily be used to interrogate action potentials with high spatial resolution, but they still have limitations in terms of signal-to-noise (S/N) ratio, pharmacological side effects, phototoxicity, and difficulty in differentiating single spikes(108). For electrical probes (Figure 5), the single electrical connection facilitates design and mechanical insertion into cells, but the requirement of direct ionic and/or electrical junctions between probe tips and cytosol also introduce several limitations. First, the tip size of these probes (~0.2 to 5 μm) is a compromise between being small enough (<5 μm) to penetrate or rupture the cell membrane with minimum damage and large enough (>0.2 μm) to yield a junction impedance that is sufficiently low so that small cellular signals can be discerned from thermal noise. Second, direct exposure of intracellular species to extraneous probe surfaces or electrolytes in probe lumen, especially for larger glass micropipettes, might induce irreversible changes to cells and, thus, prevent long-term and noninvasive cellular recordings. Finally, these probe techniques are intrinsically passive and are not capable of built-in signal processing and facile integration with other circuitries, especially given the emerging need to enable a cell-machine communication(111–114).

NanoFETs can function in a sub–10-nm-size regime(2). In principle, their exceptionally small size enables them to function as mechanically noninvasive probes capable of entering cells through endocytic pathways, as can occur with nanoparticles(115–118). Moreover, when interfacing with cells, nanoFETs process input/output information without the need for direct exchange with cellular ions; thus, the issues of interfacial impedance and biochemical invasiveness to cells can be ignored or minimized (Figure 5). In addition, because signals are transduced by change in field/potential at well-isolated surfaces, nanoFETs can detect cellular potential, as well as biological macromolecules, and could be integrated for potential multiplexed intracellular measurements. Until recently, these advantages could not be exploited, although our recent work(31,67–69) (Figure 4a) has now shown three solutions that open up these exciting opportunities.

4.3. Designs and implementation of intracellular nanoFET probes

In 2010, the first nanoFET intracellular probes were designed and chemically synthesized without lithography to encode a ~ 100 nm FET device at the apex of a kinked nanowire(31) (Figure 4a,b). This was achieved through control over cis-/trans- conformations and modulation doping during the silicon nanowire synthesis(31,32). Subsequently, the free arms of such kinked nanowires were electrically contacted to free-standing and flexible electrodes. Electrical characterization of the 3D nanowire probes showed they were robust to mechanical deformation, recorded solution pH changes with high-resolution, and, when modified with phospholipid bilayers, recorded the intracellular potential of single cells. Significantly, electrical recordings of spontaneously beating cardiomyocytes demonstrated that the 3D nanoFET probes continuously monitored extra- to intracellular signals during cellular uptake for the first time. The nanometer size, biomimetic surface coating, and flexible 3D device geometry render these active semiconductor nanoprobes a new and powerful tool for intracellular electrophysiology.

The kinked nanoFET based intracellular recording represents the first example of interfacing semiconductor devices with cells intracellularly, but the kink configuration and device design also place certain limits on the probe size and the potential for multiplexing. To address these issues, we reported a new device platform in which a branched SiO_2 nanotube was synthetically integrated on top of a nanoFET (BIT-FET)(67)(Figure 4a,c). This branched nanotube penetrated the cell membrane, bringing the cell cytosol into contact with the extracellular FET, thus allowing intracellular recording of transmembrane potential. Studies of cardiomyocyte cells demonstrated that when phospholipid-modified BIT-FETs are brought close to cells, the nanotubes spontaneously penetrate the cell membrane and yield full-amplitude intracellular action potentials, thus showing that a stable and tight seal forms between the nanotube and cell membrane. Significantly, we also showed that multiple BIT-FETs can be used for multiplexed intracellular electrical recordings from both single cells and networks of cells.

Recently, we also demonstrated a conceptually new and practically simple nanoFET probe that consists of a single semiconductor nanotube(68)(Figure 4a,d). The fabrication of the active nanotube transistor (ANTT) intracellular probe involves the fabrication of S/D contacts to one end of a silicon or other semiconductor nanotube, and electrical isolation of these S/D contacts from surrounding medium. Then the solution filling the interior of the nanotube can gate the transistor and the variation of interior electrochemical potential is recorded as a change in device conductance. In experiments, the free end of ANTT probes were inserted into cardiomyocyte cells, and the time-dependent changes associated with action potential spikes were recorded by this nanoFET probe. As expected, if a similarly configured solid nanowire nanoFET was inserted into the cell, no signal was observed since it would not be possible to “gate” the nanoFET. Finally, the straightforward fabrication of ANTT devices was exploited to prepare multiple ANTTs at the end of single probes, which enabled multiplexed recording of full-amplitude intracellular action potentials from single cells, and multiplexed arrays of single ANTT device probes (Figure 4d).

4.4. Challenges and promises

Despite these advances, additional work remains to advance further the nanoFET-based intracellular measurement techniques (Figure 5). For example, the S/N is, at current stage, not better than that from glass micropipette recordings although spatial resolution is much higher. The current designs of nanoFETs only enable potential recordings, but measurement of ionic currents is also possible if other signal transduction mechanisms are combined with nanoFET. Moreover, the capability for cell stimulation in addition to recording is still lacking. Nevertheless, we believe that the advantages of the nanoFET intracellular probes

already demonstrated in our work, including the capability to realize sub-10 nm probes, ease of operations (*e.g.*, there is no need to compensate/calibrate the probe junction potential and capacitance, etc.), the biomimetic cellular entrance, minimal mechanical and biochemical invasiveness, and the potential for large-scale, high-density, multiplexed recording, make them very attractive new measurement tools that will extend substantially the scope of fundamental and applied electrophysiology studies to regimes hard to access by current methods. For example, an exciting future application of these nanoFET probes will be measuring membrane potentials directly from cellular organelles, a Holy Grail in intracellular electrophysiology.

5. NANOELECTRONICS INNERVATED SYNTHETIC TISSUES

The development of synthetic 3D macroporous biomaterials as extracellular matrices (ECMs) represents a key area because (i) functionalized 3D biomaterials allow for studies of cell/tissue development in the presence of spatiotemporal biochemical stimulants(119,120), and (ii) the understanding of pharmacological response of cells within synthetic tissues(121–123) is expected to provide a more robust link to *in vivo* disease treatment than that from 2D cell cultures. Advancing further such biomaterials requires capabilities for monitoring cells throughout the 3D microenvironment. While electrical sensors are attractive tools, it has not been possible to integrate such elements with porous 3D scaffolds for localized real-time monitoring of cellular activities and physicochemical changes.

Recent efforts in coupling electronics and tissues have focused on flexible, stretchable planar arrays that conform to tissue surfaces(10,42,53,98–101), or implantable microfabricated probes(124). These approaches have been used to probe electrical activities near surfaces of the heart, brain and skin, and they have shown translational potential. However, these new electronic tools are currently limited in merging electronics with tissues throughout 3D space while minimizing tissue disruption, because of the 2D support structures and the electronic sensors are generally much larger scale than the extracellular matrix (ECM) and cells. Our studies using nanoFETs have shown that electronic devices with nanoscopic features were able detect extra- and intracellular potentials from single cells but had also been limited to surface or near surface recording from tissue and organs(42,53). Merging electronics seamlessly throughout tissues (Figure 6a) had remained a major challenge. To address this challenge we recently set-forth the key constraints(66) include: (1) The electronic structures must be macroporous, not planar, to enable 3D interpenetration with biomaterials; (2) the electronic network should have nanometer to micrometer scale features comparable to biomaterial scaffolds; and (3) the electronic network must have 3D interconnectivity and mechanical properties similar to biomaterials (Figure 6b).

5.1. A new concept of merging electronics with cellular systems

Our fundamentally new approach integrates nanoelectronics into tissues in 3D, and the integrative synthetic approach involved stepwise incorporation of biomimetic and biological elements into nanoelectronic networks across nanometer to centimeter size scales(66) (Figure 6a). First, chemically synthesized kinked or uniform silicon nanowires were registered and electrically connected to yield FETs (step A, Figure 6a), forming the nanoelectronic sensor elements for hybrid biomaterials. Second, individual nanoFET devices were arranged and integrated into free-standing macroporous scaffolds (step B, Figure 6a), termed ‘nanoelectronic scaffolds’ (nanoES). The nanoES were tailored to be 3D, to have nanometer to micrometer features with high (>99 %) porosity, and to be highly flexible and biocompatible. NanoES could also be hybridized with biodegradable synthetic ECMs to enable suitable cellular microenvironments prior to tissue culture. Finally, cells were cultured inside nanoES or hybrid nanoES (step C, Figure 6a), with subsequent generation of biological species and the merging of cells with nanoelectronics in 3D. The entire

biomimetic process make a natural transition from electronic to biological systems by integrating the third component, nanoES, into the synthetic tissues (Figure 6c). Metal-electrode or carbon nanotube/nanofiber based passive detectors are not considered in our work because impedance limitations (*i.e.*, signal/noise and temporal resolution degrade as the area of the metal or carbon electrodes is decreased) make it difficult to reduce the size of individual electrodes to the subcellular level, a size regime necessary to achieve noninvasive 3D interface of electronics with cells in tissue.

5.2. Designs and preparation of synthetic tissues

In our experiments, we have designed two types of 3D macroporous nanoES (reticular- and mesh- nanoES) to mimic the structure of natural tissue scaffolds (Figure 7)(66). These nanoES were formed by self-organization of coplanar reticular networks with built-in strain (Figure 7a) and by manual manipulation of 2D mesh matrices (Figure 7b). We showed that nanoES exhibited robust electronic properties and could be used alone or seamlessly merged with other biomaterials as biocompatible extracellular scaffolds for efficient 3D culture of neurons, cardiomyocytes and smooth muscle cells (Figure 7c,d). Significantly, we have demonstrated multiplexed electrical recordings of extracellular field potentials from 3D nanoelectronic innervated cardiac patches, including the effects of drugs (Figure 7e,f). The results suggested the feasibility of continuous electrical monitoring of engineered tissue in 3D for *in vitro* therapeutic assays. Finally, we have used 3D distributed nanoelectronic devices for simultaneous monitoring of pH inside and outside an engineered tubular vascular construct that was developed from the nanoelectronic scaffold, suggesting the potential of a multifunctional prosthetics.

5.3. Challenges and promises

These results open up a new field whereby nanoelectronics are merged with biological systems in 3D, and as in any nascent area opportunities and challenges abound. For example, the sensing capabilities could be broadened to address various disease states, *in vitro* (organ-on-a-chip) or *in vivo*(125) by exploiting the diverse nanowire building blocks available from designed synthesis. Cell or tissue interactions with nanoES could be fine-tuned by modification with cell growth determinants(121). NanoES could be enhanced to provide electrical and mechanical stimulation to enhance cell culture; *in vivo* these properties could provide functionalities such as pacing, and moduli that match those of host tissues. Long-term *in vivo* biocompatibility of nanoES should be studied. One can envision nanoES-based tissues that are hard-wired to provide closed-loop systems that sense and treat, that enable telemetric monitoring of physiological processes, or that provide connections between engineered constructs with the host nervous system.

6. WHAT'S NEXT?

The challenges associated with nanotechnology applications in biomedical sciences are numerous, but the impact on understanding how the cardiac or nervous systems work, how they fails in disease and how we can intervene at a nanoscopic or even a molecular level is significant. For example, neural developmental factors, such as the cadherins, laminins and bone morphometric protein families, as well as their receptors, could be manipulated in new ways(126). The bottom-up nanowire nanotechnology offers the capacity to explore the functional specificity of these molecules by incorporating them into pre-defined locations in nanowire devices to have highly targeted effects towards single cells.

The merging of nanoelectronics or nanoscience in general with the entire fields of synthetic biology and/or system biology(46,47) is also tempting and could be highly rewarding. This would be one of the next big leaps in materials sciences and biological sciences. It is

especially true given that there's a whole toolbox of nanoelectronic and nanophotonic devices that one can think about building into cellular circuitry and merging them with biological information processing systems, and the fact that we have already achieved the intracellular interrogation(31) and the 3D electrical innervation of tissues(66) with semiconductor nanoelectronics!

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LITERATURE CITED

1. http://nano.gov/sites/default/files/pub_resource/nni_siginit_nanoelectronics_jul_2010.pdf
2. Ieong M, Doris B, Kedzierski J, Rim K, Yang M. Silicon device scaling to the sub-10-nm regime. Science (New York, N.Y.). 2004; 306:2057–2060.
3. Lieber CM. Semiconductor nanowires: A platform for nanoscience and nanotechnology. Mrs Bulletin. 2011; 36:1052–1063. [PubMed: 22707850]
4. Lu W, Lieber CM. Semiconductor nanowires. Journal of Physics D-Applied Physics. 2006; 39:R387–R406.
5. Lu W, Lieber CM. Nanoelectronics from the bottom up. Nature Materials. 2007; 6:841–850.
6. Lu W, Xie P, Lieber CM. Nanowire Transistor Performance Limits and Applications. Ieee Transactions on Electron Devices. 2008; 55:2859–2876.
7. Cui Y, Lieber CM. Functional nanoscale electronic devices assembled using silicon nanowire building blocks. Science. 2001; 291:851–853. [PubMed: 11157160]
8. Duan XF, Huang Y, Cui Y, Wang JF, Lieber CM. Indium phosphide nanowires as building blocks for nanoscale electronic and optoelectronic devices. Nature. 2001; 409:66–69. [PubMed: 11343112]
9. Friedman RS, McAlpine MC, Ricketts DS, Ham D, Lieber CM. High-speed integrated nanowire circuits. Nature. 2005; 434:1085. [PubMed: 15858562]
10. Rogers JA, Lagally MG, Nuzzo RG. Synthesis, assembly and applications of semiconductor nanomembranes. Nature. 2011; 477:45–53. [PubMed: 21886156]
11. Sun Y, Rogers JA. Inorganic semiconductors for flexible electronics. Advanced Materials. 2007; 19:1897–1916.
12. Yan H, Choe HS, Nam SW, Hu YJ, Das S, et al. Programmable nanowire circuits for nanoprocessors. Nature. 2011; 470:240–244. [PubMed: 21307937]
13. Choi, CL.; Alivisatos, AP. From Artificial Atoms to Nanocrystal Molecules: Preparation and Properties of More Complex Nanostructures. In: Leone, SR.; Cremer, PS.; Groves, JT.; Johnson, MA.; Richmond, G., editors. Annual Review of Physical Chemistry. Vol. Vol 61. 2010. p. 369-389.
14. Yin Y, Alivisatos AP. Colloidal nanocrystal synthesis and the organic-inorganic interface. Nature. 2005; 437:664–670. [PubMed: 16193041]
15. Law M, Goldberger J, Yang PD. Semiconductor nanowires and nanotubes. Annual Review of Materials Research. 2004; 34:83–122.
16. Li Y, Qian F, Xiang J, Lieber CM. Nanowire electronic and optoelectronic devices. Materials Today. 2006; 9:18–27.
17. Bonaccorso F, Sun Z, Hasan T, Ferrari AC. Graphene photonics and optoelectronics. Nature Photonics. 2010; 4:611–622.
18. Geim AK. Graphene: Status and Prospects. Science. 2009; 324:1530–1534. [PubMed: 19541989]
19. Kim K, Choi JY, Kim T, Cho SH, Chung HJ. A role for graphene in silicon-based semiconductor devices. Nature. 2011; 479:338–344. [PubMed: 22094694]
20. Schwierz F. Graphene transistors. Nature Nanotechnology. 2010; 5:487–496.
21. Bjork MT, Ohlsson BJ, Sass T, Persson AI, Thelander C, et al. One-dimensional heterostructures in semiconductor nanowhiskers. Applied Physics Letters. 2002; 80:1058–1060.

22. Gudiksen MS, Lauhon LJ, Wang J, Smith DC, Lieber CM. Growth of nanowire superlattice structures for nanoscale photonics and electronics. Nature. 2002; 415:617–620. [PubMed: 11832939]
23. Yang C, Zhong ZH, Lieber CM. Encoding electronic properties by synthesis of axial modulation-doped silicon nanowires. Science. 2005; 310:1304–1307. [PubMed: 16311329]
24. Jiang XC, Tian BZ, Xiang J, Qian F, Zheng GF, et al. Rational growth of branched nanowire heterostructures with synthetically encoded properties and function. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108:12212–12216. [PubMed: 21730174]
25. Lauhon LJ, Gudiksen MS, Wang CL, Lieber CM. Epitaxial core-shell and core-multishell nanowire heterostructures. Nature. 2002; 420:57–61. [PubMed: 12422212]
26. Dick KA, Deppert K, Larsson MW, Martensson T, Seifert W, et al. Synthesis of branched 'nanotrees' by controlled seeding of multiple branching events. Nature Materials. 2004; 3:380–384.
27. Algra RE, Verheijen MA, Borgstrom MT, Feiner LF, Immink G, et al. Twinning superlattices in indium phosphide nanowires. Nature. 2008; 456:369–372. [PubMed: 19020617]
28. Caroff P, Dick KA, Johansson J, Messing ME, Deppert K, Samuelson L. Controlled polytypic and twin-plane superlattices in III-V nanowires. Nature Nanotechnology. 2009; 4:50–55.
29. Tian BZ, Zheng XL, Kempa TJ, Fang Y, Yu NF, et al. Coaxial silicon nanowires as solar cells and nanoelectronic power sources. Nature. 2007; 449:U885–U888.
30. Schwarz KW, Tersoff J, Kodambaka S, Chou YC, Ross FM. Geometrical Frustration in Nanowire Growth. Physical Review Letters. 2011; 107
31. Tian BZ, Cohen-Karni T, Qing Q, Duan XJ, Xie P, Lieber CM. Three-Dimensional, Flexible Nanoscale Field-Effect Transistors as Localized Bioprobes. Science. 2010; 329:830–834. [PubMed: 20705858]
32. Tian BZ, Xie P, Kempa TJ, Bell DC, Lieber CM. Single-crystalline kinked semiconductor nanowire superstructures. Nature Nanotechnology. 2009; 4:824–829.
33. Cohen-Karni T, Timko BP, Weiss LE, Lieber CM. Flexible electrical recording from cells using nanowire transistor arrays. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106:7309–7313. [PubMed: 19365078]
34. Fan Z, Ho JC, Jacobson ZA, Razavi H, Javey A. Large-scale, heterogeneous integration of nanowire arrays for image sensor circuitry. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105:11066–11070. [PubMed: 18685094]
35. Fan Z, Ho JC, Takahashi T, Yerushalmi R, Takei K, et al. Toward the Development of Printable Nanowire Electronics and Sensors. Advanced Materials. 2009; 21:3730–3743.
36. Goldberger J, Hochbaum AI, Fan R, Yang P. Silicon vertically integrated nanowire field effect transistors. Nano Letters. 2006; 6:973–977.
37. Javey A, Nam S, Friedman RS, Yan H, Lieber CM. Layer-by-layer assembly of nanowires for three-dimensional, multifunctional electronics. Nano Letters. 2007; 7:773–777. [PubMed: 17266383]
38. McAlpine MC, Friedman RS, Jin S, Lin KH, Wang WU, Lieber CM. High-performance nanowire electronics and photonics on glass and plastic substrates. Nano Letters. 2003; 3:1531–1535.
39. McAlpine MC, Friedman RS, Lieber CM. High-performance nanowire electronics and photonics and nanoscale patterning on flexible plastic substrates. Proceedings of the Ieee. 2005; 93:1357–1363.
40. Nam S, Jiang XC, Xiong QH, Ham D, Lieber CM. Vertically integrated, three-dimensional nanowire complementary metal-oxide-semiconductor circuits. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106:21035–21038. [PubMed: 19940239]
41. Qing Q, Pal SK, Tian B, Duan X, Timko BP, et al. Nanowire transistor arrays for mapping neural circuits in acute brain slices. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107:1882–1887. [PubMed: 20133836]

42. Timko BP, Cohen-Karni T, Yu GH, Qing Q, Tian BZ, Lieber CM. Electrical Recording from Hearts with Flexible Nanowire Device Arrays. Nano Letters. 2009; 9:914–918. [PubMed: 19170614]
43. Whang D, Jin S, Wu Y, Lieber CM. Large-scale hierarchical organization of nanowire arrays for integrated nanosystems. Nano Letters. 2003; 3:1255–1259.
44. Yu GH, Cao AY, Lieber CM. Large-area blown bubble films of aligned nanowires and carbon nanotubes. Nature Nanotechnology. 2007; 2:372–377.
45. Yu GH, Li XL, Lieber CM, Cao AY. Nanomaterial-incorporated blown bubble films for large-area, aligned nanostructures. Journal of Materials Chemistry. 2008; 18:728–734.
46. Nandagopal N, Elowitz MB. Synthetic Biology: Integrated Gene Circuits. Science. 2011; 333:1244–1248. [PubMed: 21885772]
47. Ruder WC, Lu T, Collins JJ. Synthetic Biology Moving into the Clinic. Science. 2011; 333:1248–1252. [PubMed: 21885773]
48. Slusarczyk AL, Lin A, Weiss R. Foundations for the design and implementation of synthetic genetic circuits. Nature Reviews Genetics. 2012; 13:406–420.
49. Weber W, Fussenegger M. Emerging biomedical applications of synthetic biology. Nature Reviews Genetics. 2012; 13:21–35.
50. Khalil AS, Collins JJ. Synthetic biology: applications come of age. Nature Reviews Genetics. 2010; 11:367–379.
51. Mukherji S, van Oudenaarden A. Synthetic biology: understanding biological design from synthetic circuits. Nature Reviews Genetics. 2009; 10:859–871.
52. Patolsky F, Timko BP, Zheng G, Lieber CM. Nanowire-based nanoelectronic devices in the life sciences. Mrs Bulletin. 2007; 32:142–149.
53. Timko BP, Cohen-Karni T, Qing Q, Tian BZ, Lieber CM. Design and Implementation of Functional Nanoelectronic Interfaces With Biomolecules, Cells, and Tissue Using Nanowire Device Arrays. Ieee Transactions on Nanotechnology. 2010; 9:269–280. [PubMed: 21785576]
54. Fan ZY, Razavi H, Do JW, Moriwaki A, Ergen O, et al. Three-dimensional nanopillar-array photovoltaics on low-cost and flexible substrates. Nature Materials. 2009; 8:648–653.
55. Boettcher SW, Spurgeon JM, Putnam MC, Warren EL, Turner-Evans DB, et al. Energy-Conversion Properties of Vapor-Liquid-Solid-Grown Silicon Wire-Array Photocathodes. Science. 2010; 327:185–187. [PubMed: 20056886]
56. Kelzenberg MD, Boettcher SW, Petykiewicz JA, Turner-Evans DB, Putnam MC, et al. Enhanced absorption and carrier collection in Si wire arrays for photovoltaic applications. Nature Materials. 2010; 9:239–244.
57. Garnett E, Yang PD. Light Trapping in Silicon Nanowire Solar Cells. Nano Letters. 2010; 10:1082–1087. [PubMed: 20108969]
58. Qin Y, Wang XD, Wang ZL. Microfibre-nanowire hybrid structure for energy scavenging. Nature. 2008; 451:U809–U805.
59. Xu S, Qin Y, Xu C, Wei YG, Yang RS, Wang ZL. Self-powered nanowire devices. Nature Nanotechnology. 2010; 5:366–373.
60. Wang XD, Song JH, Liu J, Wang ZL. Direct-current nanogenerator driven by ultrasonic waves. Science. 2007; 316:102–105. [PubMed: 17412957]
61. Wang ZL, Song JH. Piezoelectric nanogenerators based on zinc oxide nanowire arrays. Science. 2006; 312:242–246. [PubMed: 16614215]
62. Kim W, Ng JK, Kunitake ME, Conklin BR, Yang PD. Interfacing silicon nanowires with mammalian cells. Journal of the American Chemical Society. 2007; 129:7228. + [PubMed: 17516647]
63. Chevrier N, Mertins P, Artyomov MN, Shalek AK, Iannacone M, et al. Systematic Discovery of TLR Signaling Components Delineates Viral-Sensing Circuits. Cell. 2011; 147:853–867. [PubMed: 22078882]
64. Shalek AK, Robinson JT, Karp ES, Lee JS, Ahn DR, et al. Vertical silicon nanowires as a universal platform for delivering biomolecules into living cells. Proceedings of the National

Academy of Sciences of the United States of America. 2010; 107:1870–1875. [PubMed: 20080678]

65. Xie C, Hanson L, Cui Y, Cui BX. Vertical nanopillars for highly localized fluorescence imaging. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108:3894–3899. [PubMed: 21368157]
66. Tian, BZ.; Liu, J.; Dvir, T.; Jin, LH.; Tsui, JH., et al. Nature Materials. 2012. Macroporous nanowire nanoelectronic scaffolds for synthetic tissues. published online:26 August 2012|
67. Duan XJ, Gao RX, Xie P, Cohen-Karni T, Qing Q, et al. Intracellular recordings of action potentials by an extracellular nanoscale field-effect transistor. Nature Nanotechnology. 2012; 7:174–179.
68. Gao RX, Strehle S, Tian BZ, Cohen-Karni T, Xie P, et al. Outside Looking In: Nanotube Transistor Intracellular Sensors. Nano Letters. 2012; 12:3329–3333. [PubMed: 22583370]
69. Jiang Z, Qing Q, Xie P, Gao RX, Lieber CM. Kinked p-n Junction Nanowire Probes for High Spatial Resolution Sensing and Intracellular Recording. Nano Letters. 2012; 12:1711–1716. [PubMed: 22309132]
70. Sakmann B, Neher E. Patch clamp techniques for studying ionic channels in excitable-membranes. Annual Review of Physiology. 1984; 46:455–472.
71. Robinson JT, Jorgolli M, Shalek AK, Yoon MH, Gertner RS, Park H. Vertical nanowire electrode arrays as a scalable platform for intracellular interfacing to neuronal circuits. Nature Nanotechnology. 2012; 7:180–184.
72. Ewing AG, Strein TG, Lau YY. Analytical chemistry in microenvironments-single nerve cells. Accounts of Chemical Research. 1992; 25:440–447.
73. Schrlau MG, Dun NJ, Bau HH. Cell Electrophysiology with Carbon Nanopipettes. Acs Nano. 2009; 3:563–568. [PubMed: 19309170]
74. Xie C, Lin ZL, Hanson L, Cui Y, Cui BX. Intracellular recording of action potentials by nanopillar electroporation. Nature Nanotechnology. 2012; 7:185–190.
75. Bohn PW. Nanoscale Control and Manipulation of Molecular Transport in Chemical Analysis. In Annual Review of Analytical Chemistry. 2009:279–296.
76. Henstridge MC, Compton RG. Mass Transport to micro- and nanoelectrodes and their arrays: a review. Chemical Record. 2012; 12:63–71. [PubMed: 22144415]
77. Walsh DA, Lovelock KRJ, Licence P. Ultramicroelectrode voltammetry and scanning electrochemical microscopy in room-temperature ionic liquid electrolytes. Chemical Society Reviews. 2010; 39:4185–4194. [PubMed: 20835469]
78. Yeh JI, Shi HB. Nanoelectrodes for biological measurements. Wiley Interdisciplinary Reviews-Nanomedicine and Nanobiotechnology. 2010; 2:176–188. [PubMed: 20073052]
79. Sze, SM. Physics of semiconductor devices. 2 edition. Wiley-Interscience; 1981. p. 880
80. Buzsaki G, Anastassiou CA, Koch C. The origin of extracellular fields and currents - EEG, ECoG, LFP and spikes. Nature Reviews Neuroscience. 2012; 13:407–420.
81. Plonsey, R.; Barr, RC. Bioelectricity-a quantitative approach. 2 edition. Kluwer academic/Plenum publishers; 2000.
82. Givargizov EI. Fundamental aspects of VLS growth. Journal of Crystal Growth. 1975; 31:20–30.
83. Wagner RS, Ellis WC. Vapor-liquid-solid mechanism of single crystal growth. Applied Physics Letters. 1964; 4:89. -&.
84. Cui Y, Lauhon LJ, Gudiksen MS, Wang JF, Lieber CM. Diameter-controlled synthesis of single-crystal silicon nanowires. Applied Physics Letters. 2001; 78:2214–2216.
85. Morales AM, Lieber CM. A laser ablation method for the synthesis of crystalline semiconductor nanowires. Science. 1998; 279:208–211. [PubMed: 9422689]
86. Duan XF, Lieber CM. General synthesis of compound semiconductor nanowires. Advanced Materials. 2000; 12:298–302.
87. Duan XF, Lieber CM. Laser-assisted catalytic growth of single crystal GaN nanowires. Journal of the American Chemical Society. 2000; 122:188–189.

88. Cohen-Karni T, Casanova D, Cahoon JF, Qing Q, Bell DC, Lieber CM. Synthetically Encoded Ultrashort-Channel Nanowire Transistors for Fast, Pointlike Cellular Signal Detection. Nano Letters. 2012; 12:2639–2644. [PubMed: 22468846]
89. Lieber CM. Nanowire superlattices. Nano Letters. 2002; 2:81–82.
90. Wu YY, Fan R, Yang PD. Block-by-block growth of single-crystalline Si/SiGe superlattice nanowires. Nano Letters. 2002; 2:83–86.
91. Qian F, Gradecak S, Li Y, Wen CY, Lieber CM. Core/multishell nanowire heterostructures as multicolor, high-efficiency light-emitting diodes. Nano Letters. 2005; 5:2287–2291. [PubMed: 16277469]
92. Hu YJ, Churchill HOH, Reilly DJ, Xiang J, Lieber CM, Marcus CM. A Ge/Si heterostructure nanowire-based double quantum dot with integrated charge sensor. Nature Nanotechnology. 2007; 2:622–625.
93. Hu YJ, Kuemmeth F, Lieber CM, Marcus CM. Hole spin relaxation in Ge-Si core-shell nanowire qubits. Nature Nanotechnology. 2012; 7:47–50.
94. Lu W, Xiang J, Timko BP, Wu Y, Lieber CM. One-dimensional hole gas in germanium/silicon nanowire heterostructures. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102:10046–10051. [PubMed: 16006507]
95. Xiang J, Lu W, Hu YJ, Wu Y, Yan H, Lieber CM. Ge/Si nanowire heterostructures as high-performance field-effect transistors. Nature. 2006; 441:489–493. [PubMed: 16724062]
96. Wang D, Qian F, Yang C, Zhong ZH, Lieber CM. Rational growth of branched and hyperbranched nanowire structures. Nano Letters. 2004; 4:871–874.
97. Zhou X, Moran-Mirabal JM, Craighead HG, McEuen PL. Supported lipid bilayer/carbon nanotube hybrids. Nature Nanotechnology. 2007; 2:185–190.
98. Kim D-H, Lu N, Ghaffari R, Kim Y-S, Lee SP, et al. Materials for multifunctional balloon catheters with capabilities in cardiac electrophysiological mapping and ablation therapy. Nature Materials. 2011; 10:316–323.
99. Viventi J, Kim D-H, Moss JD, Kim Y-S, Blanco JA, et al. A Conformal, Bio-Interfaced Class of Silicon Electronics for Mapping Cardiac Electrophysiology. Science Translational Medicine. 2010; 2
100. Viventi J, Kim D-H, Vigeland L, Frechette ES, Blanco JA, et al. Flexible, foldable, actively multiplexed, high-density electrode array for mapping brain activity in vivo. Nature Neuroscience. 2011; 14:U1599–U1138.
101. Kim DH, Viventi J, Amsden JJ, Xiao JL, Vigeland L, et al. Dissolvable films of silk fibroin for ultrathin conformal bio-integrated electronics. Nature Materials. 2010; 9:511–517.
102. Hu YJ, Xiang J, Liang GC, Yan H, Lieber CM. Sub-100 nanometer channel length Ge/Si nanowire transistors with potential for 2 THz switching speed. Nano Letters. 2008; 8:925–930. [PubMed: 18251518]
103. Patolsky F, Timko BP, Yu GH, Fang Y, Greytak AB, et al. Detection, stimulation, and inhibition of neuronal signals with high-density nanowire transistor arrays. Science. 2006; 313:1100–1104. [PubMed: 16931757]
104. Mercanzini A, Colin P, Bensadoun JC, Bertsch A, Renaud P. In Vivo Electrical Impedance Spectroscopy of Tissue Reaction to Microelectrode Arraysl. Ieee Transactions on Biomedical Engineering. 2009; 56:1909–1918. [PubMed: 19362904]
105. Patrick E, Orazem ME, Sanchez JC, Nishida T. Corrosion of tungsten microelectrodes used in neural recording applications. Journal of Neuroscience Methods. 2011; 198:158–171. [PubMed: 21470563]
106. Chernomordik LV, Kozlov MM. Mechanics of membrane fusion. Nature Structural & Molecular Biology. 2008; 15:675–683.
107. Kauer JS, White J. Imaging and coding in the olfactory system. Annual Review of Neuroscience. 2001; 24:963–979.
108. Grinvald A, Hildesheim R. VSDI: A new era in functional imaging of cortical dynamics. Nature Reviews Neuroscience. 2004; 5:874–885.

109. Kralj JM, Douglass AD, Hochbaum DR, Maclaurin D, Cohen AE. Optical recording of action potentials in mammalian neurons using a microbial rhodopsin. Nature Methods. 2012; 9:U90–U130.
110. Kralj JM, Hochbaum DR, Douglass AD, Cohen AE. Electrical Spiking in Escherichia coli Probed with a Fluorescent Voltage-Indicating Protein. Science. 2011; 333:345–348. [PubMed: 21764748]
111. Hochberg LR, Bacher D, Jarosiewicz B, Masse NY, Simeral JD, et al. Reach and grasp by people with tetraplegia using a neurally controlled robotic arm. Nature. 2012; 485:U372–U121.
112. Hochberg LR, Serruya MD, Friehs GM, Mukand JA, Saleh M, et al. Neuronal ensemble control of prosthetic devices by a human with tetraplegia. Nature. 2006; 442:164–171. [PubMed: 16838014]
113. Serruya MD, Hatsopoulos NG, Paninski L, Fellows MR, Donoghue JP. Instant neural control of a movement signal. Nature. 2002; 416:141–142. [PubMed: 11894084]
114. Truccolo W, Hochberg LR, Donoghue JP. Collective dynamics in human and monkey sensorimotor cortex: predicting single neuron spikes. Nature Neuroscience. 2010; 13:U105–U275.
115. Ferrari M. Beyond drug delivery. Nature Nanotechnology. 2008; 3:131–132.
116. Nel AE, Madler L, Velegol D, Xia T, Hoek EMV, et al. Understanding biophysicochemical interactions at the nano-bio interface. Nature Materials. 2009; 8:543–557.
117. Rajendran L, Knolker HJ, Simons K. Subcellular targeting strategies for drug design and delivery. Nature Reviews Drug Discovery. 2010; 9:29–42.
118. Summers HD, Rees P, Holton MD, Brown MR, Chappell SC, et al. Statistical analysis of nanoparticle dosing in a dynamic cellular system. Nature Nanotechnology. 2011; 6:170–174.
119. Wylie RG, Ahsan S, Aizawa Y, Maxwell KL, Morshead CM, Shoichet MS. Spatially controlled simultaneous patterning of multiple growth factors in three-dimensional hydrogels. Nature Materials. 2011; 10:799–806.
120. Kloxin AM, Kasko AM, Salinas CN, Anseth KS. Photodegradable Hydrogels for Dynamic Tuning of Physical and Chemical Properties. Science. 2009; 324:59–63. [PubMed: 19342581]
121. Dvir T, Timko BP, Kohane DS, Langer R. Nanotechnological strategies for engineering complex tissues. Nature Nanotechnology. 2011; 6:13–22.
122. Hutmacher DW. Biomaterials offer cancer research the third dimension. Nature Materials. 2011; 9:90–93.
123. Prestwich GD. Evaluating drug efficacy and toxicology in three dimensions: Using synthetic extracellular matrices in drug discovery. Accounts of Chemical Research. 2008; 41:139–148. [PubMed: 17655274]
124. Prohaska OJ, Olcaytug F, Pfundner P, Dragaun H. Thin-film multiple electrode probes-possibilities and limitations. Ieee Transactions on Biomedical Engineering. 1986; 33:223–229. [PubMed: 3957371]
125. Huh D, Matthews BD, Mammoto A, Montoya-Zavala M, Hsin HY, Ingber DE. Reconstituting Organ-Level Lung Functions on a Chip. Science. 2010; 328:1662–1669. [PubMed: 20576885]
126. Silva GA. Neuroscience nanotechnology: Progress, opportunities and challenges. Nature Reviews Neuroscience. 2006; 7:65–74.

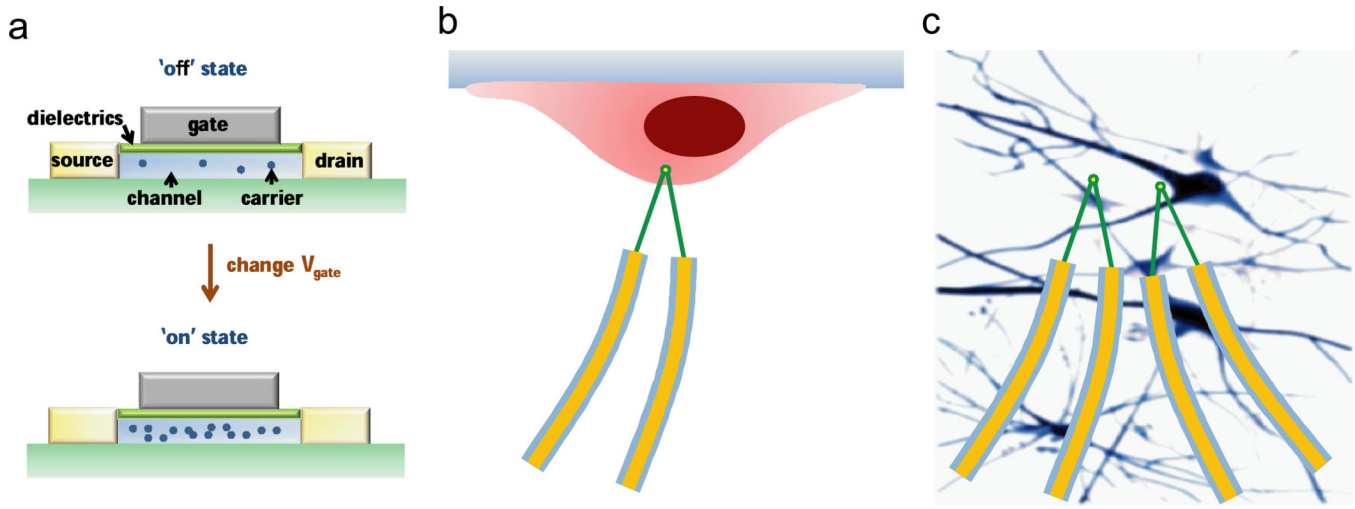


Figure 1. FET basics and electrical interfaces between nanoFET and biological systems

(*a*) Schematic of a planar FET device. In FET, current flows along a semiconductor path called the channel. At one end of the channel, there is an electrode called the source. At the other end of the channel, there is an electrode called the drain. The third electrode that applies a voltage to the channel is called gate, which modulates the electron/hole carrier density and the output of the FET devices. A small voltage change in gate signal can cause a large variation in the current from the source to the drain. This is how FET works and in particular, amplifies signals. (*b*-*c*) Schematics of electrically based cellular sensing using a kinked nanoFET, where intracellular potentials (*b*) or extracellular field potentials (*c*) can be used to change the nanoFET conductace, analogous to applying a voltage using a gate electrode.

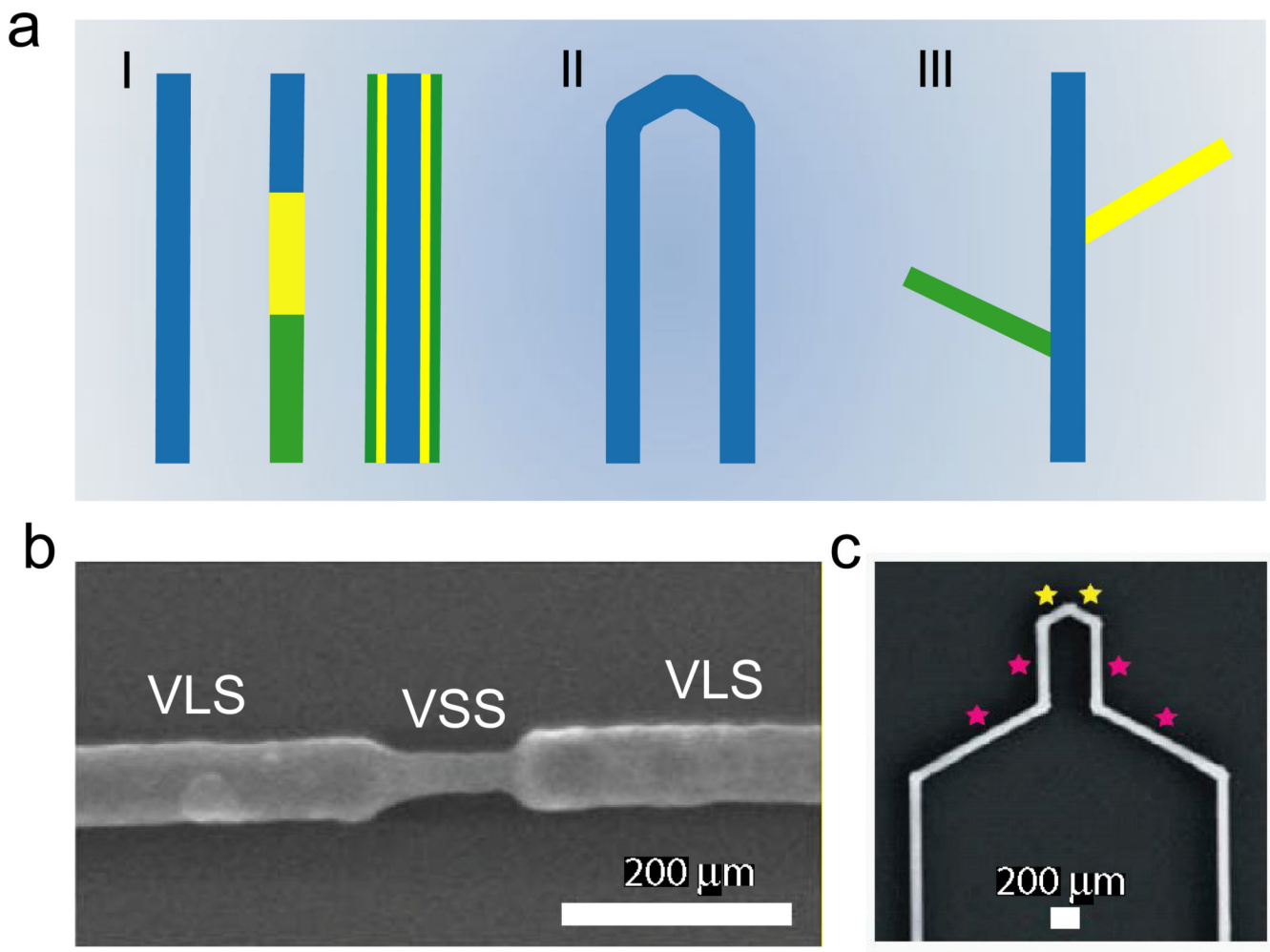


Figure 2. Semiconductor nanowire structural motifs for nanoFETs

(*a*) Schematics of 1D (I), 2D (II) and 3D (III) motifs. 1D motif (I) can have uniform composition and doping (I, left) or axially (I, middle) or radially (I, right) modulated. A kinked nanowire with structurally coherent "kinks" introduced in a controlled manner during axial elongation represents an example of 2D motif (II). Heterobranched nanowires yield 3D structure (III) and the branch junction (*e.g.*, blue/yellow segment junction) can be exploited for localized sensing. (*b*) An axial nanowire heterostructure made by modulation in VLS/VSS growth mechanisms. (*c*) A multiply kinked nanowire showing a probe structure. Yellow and magenta stars denote *cis*- and *trans*- conformations, respectively.

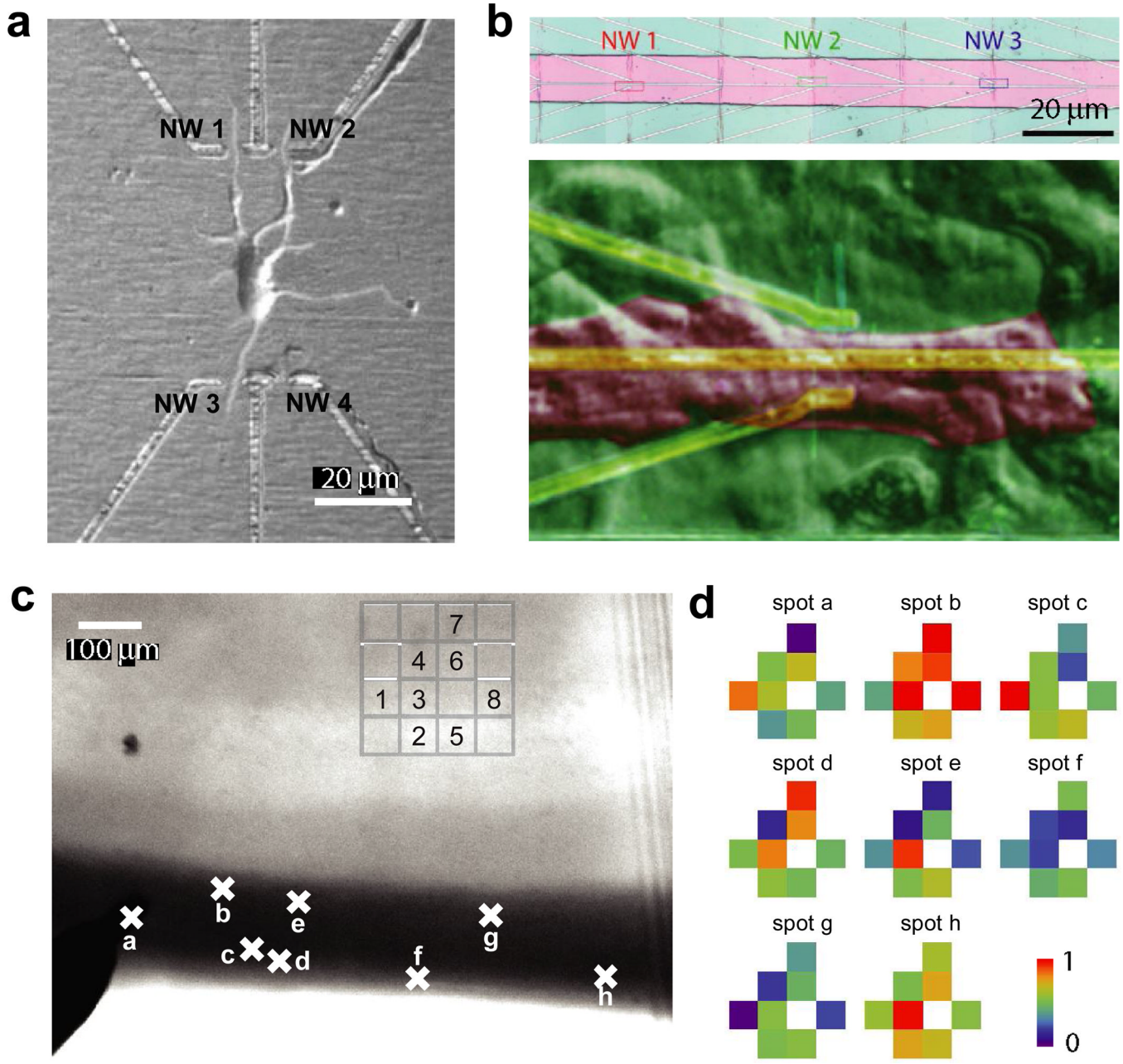


Figure 3. Multiplexed extracellular electrical recordings using nanoFETs

(*a*) Optical image of a cortical neuron interfaced to three of the four functional nanoFETs in an array. (*b*) upper panel, optical micrograph showing three nanoFET devices (NW1, NW2, and NW3) in a linear array, where pink indicates the area with exposed NW devices. Lower panel, a differential interference contrast bright field image showing individual cardiomyocytes (purple) and single nanoFETs (yellow). (*c*) Optical image of an acute slice over a 4 × 4 NWFET array. Signals were recorded simultaneously from the eight devices indicated on the image. Crosses along the LOT fiber region of the slice mark the stimulation spots *a–h*. The stimulator insertion depth was not controlled precisely in these experiments. (d) Maps of the relative signal intensity or activity for devices 1–8.

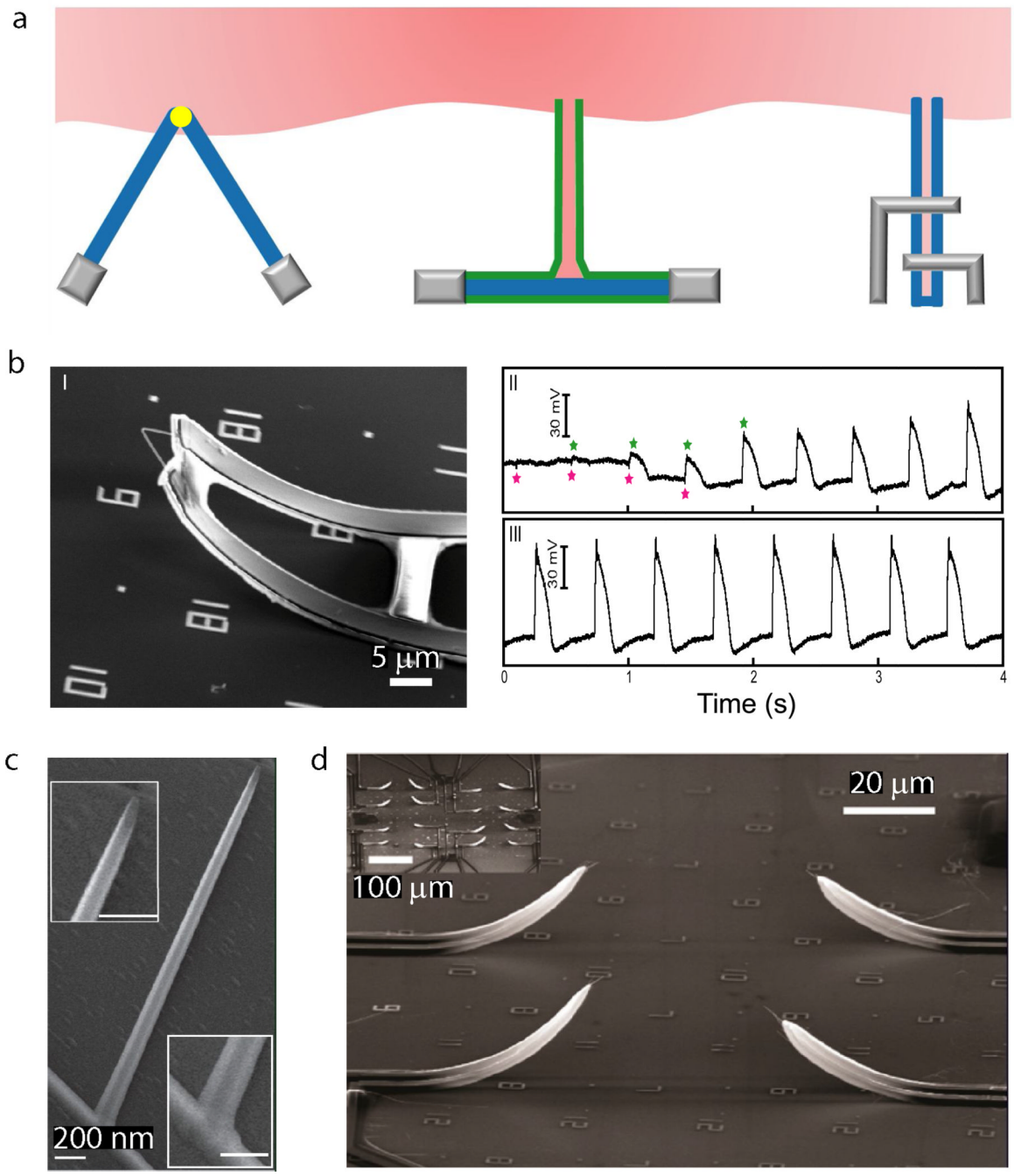
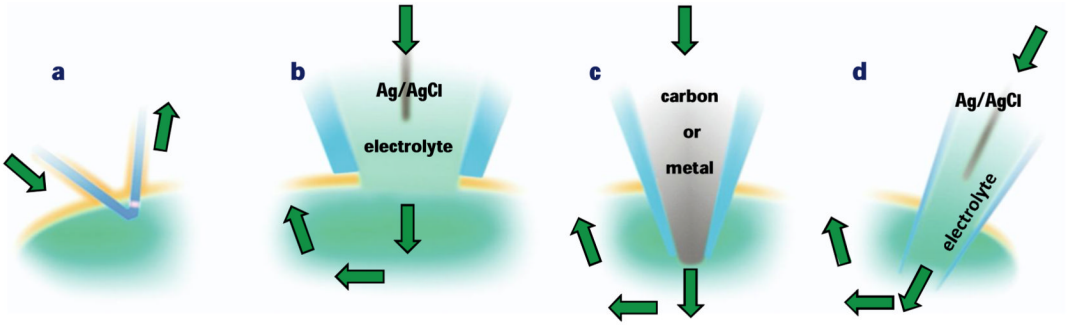


Figure 4. Intracellular electrical recordings using nanoFETs

(*a*) Schematics of kinked nanoFET (left), BIT-FET (middle) and ANTT (right) probes. (*b*) SEM image of a kinked nanoFET probe (I) and its intracellular electrical recordings (II, III) from spontaneously beating cardiomyocytes. (*c*) SEM of a BIT-FET probe, insets highlight the tip and root parts of the hollow branch. (*d*) SEM image of ANTT probe array.



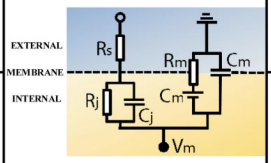
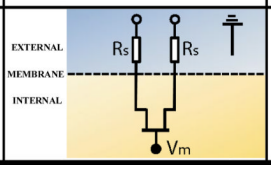
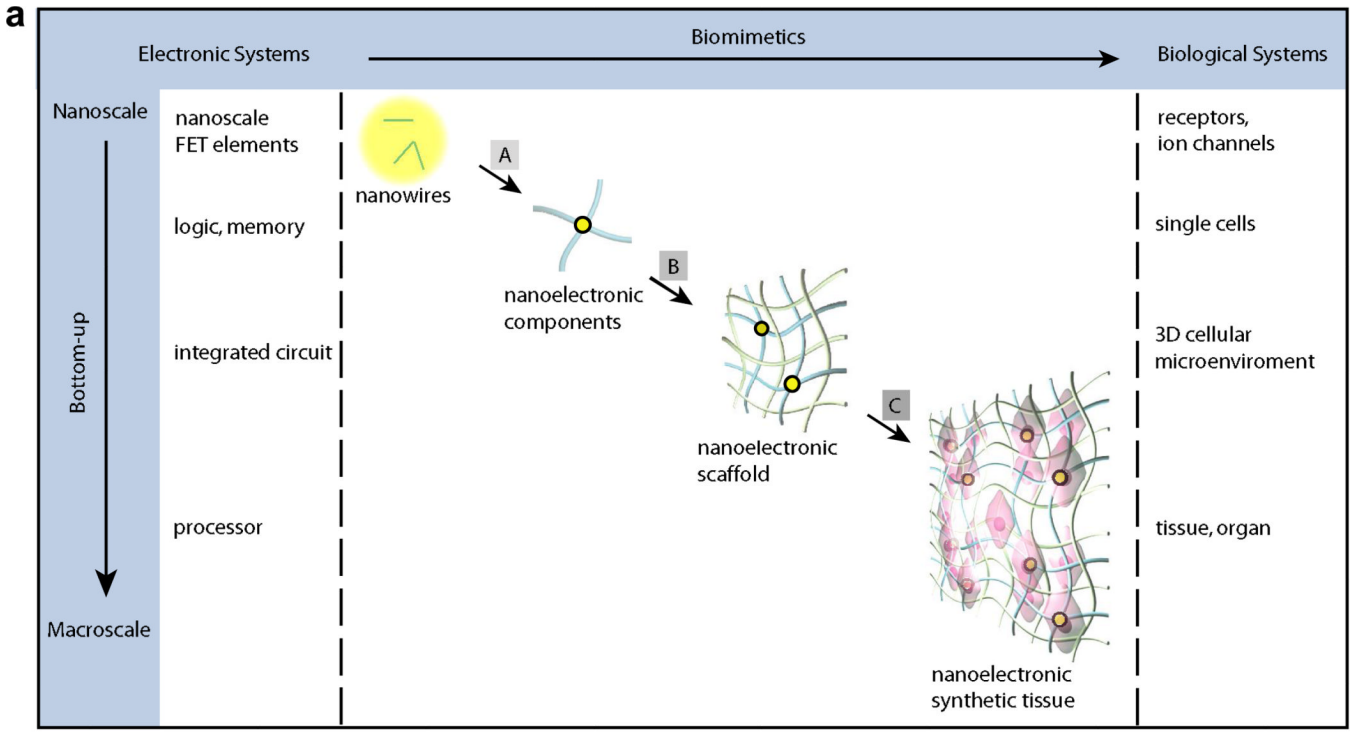
IC technique	Equivalent circuit	Size (nm)	Calibrations	Capabilities	Invasiveness	Cellular entrance
Glass micropipette (b and d)		~ 50-5000 Impedance limited	Both amplitude and shape	Can record both current and voltage, Single ion channel to whole cell recording	Electrochemical and mechanical	Mechanical or electrical
Carbon or metal micro-/nano-electrode (c)		~500-1000 Impedance limited	Both amplitude and shape	Can record both current and voltage, Whole cell recording	Electrochemical and mechanical	Mechanical or electrical
nanoFET (a)		~10-100	Amplitude	Can only record voltage, Whole cell recording, Multiplexing is scalable, High spatiotemporal resolutions	Minimal	Biological

Figure 5. A comparison between kinked nanoFET probe (a) and conventional intracellular tools (b–d)

The green arrows in (a–d) indicate the current flows. R_s, series resistance; R_j, junction resistance; R_m, membrane resistance; V_m, intracellular potential; C_j, junction capacitance; C_m, membrane capacitance.



b

Biomimetics	Targets	Approaches	Implementation Examples	Steps		
	Composition	+ Synthetic polymer	Deposit PLGA, alginate	A	B	
		+ Natural polymer	Add or secrete collagen, polysaccharides		B	C
		+ Cellular species	Biosynthesize proteins and lipids			C
		- Electronic materials	Reduce silicon, metals, dielectrics	A	B	
	Structure	+ Porosity	Increase to > 90%	A	B	
		- Feature size	Decrease to sub-micrometer	A	B	
	Mechanics	+ Flexibility	Decrease bending stiffness to < 10 nN·m	A	B	C
		+ Stretchability	Use serpentine device designs	A	B	C
	Bio-functions	+ Morphogenesis	Enable cell growth and proliferation			C
		+ Other Signalings	Activate ECM cues		B	C
		- Cytotoxicities	Use biocompatible materials	A	B	

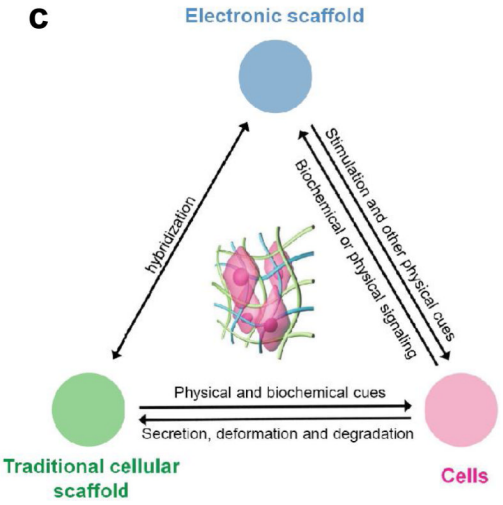


Figure 6. Integrating nanoelectronics with cells and tissue

Conventional bulk electronics are distinct from biological systems in composition, structural hierarchy, mechanics and function. Their electrical coupling at the tissue/organ level is usually limited to the tissue surface, where only boundary or global information can be gleaned unless invasive approaches are used. (*a*) A new concept was introduced where an integrated system can be created from discrete electronic and biological building blocks (for example, semiconductor nanowires, molecular precursors of polymers and single cells). Three biomimetic and bottom-up steps have been designed: step A, patterning, metallization and epoxy passivation for single-nanowire FETs; step B, forming 3D nanowire FET matrices (nanoelectric scaffolds) by self or manual organization and hybridization with traditional ECMs; step C, incorporation of cells and growth of synthetic tissue through biological processes. Yellow dots: nanowire components; blue ribbons: metal and epoxy

interconnects; green ribbons: traditional ECMs; pink: cells. (*b*) Rationale and approaches for biomimetic implementation of nanoelectronics innervated synthetic tissues. A, B and C are the same steps used in (*a*). (*c*) The new electronic scaffold component in synthetic tissues enables additional interactions with traditional cellular scaffold and cells.

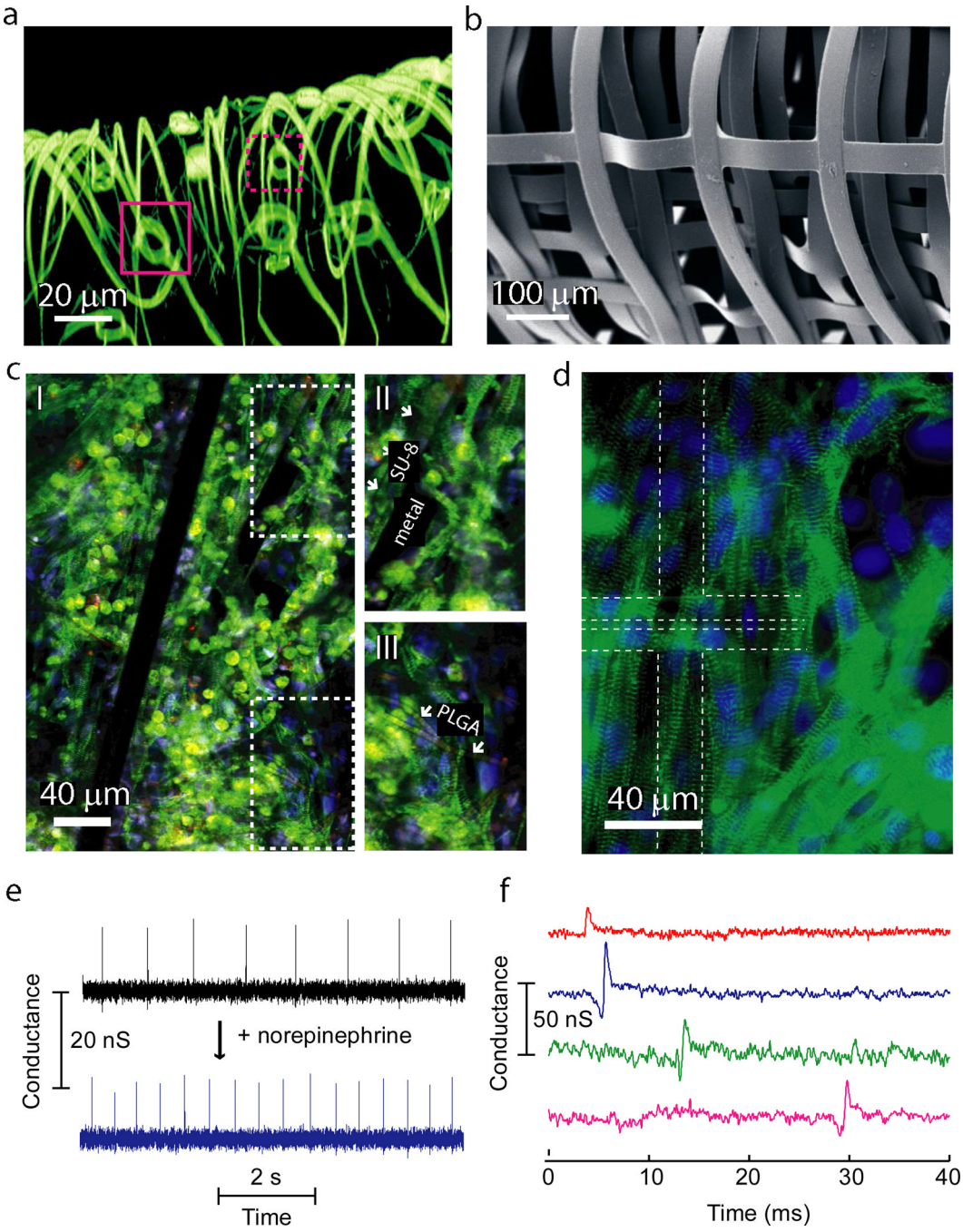


Figure 7. NanoES and synthetic tissues

(*a*) 3D reconstructed confocal fluorescence micrographs of reticular nanoES. The scaffold was labelled with rhodamine 6G. Solid and dashed open magenta squares indicate two nanowire FET devices located on different planes. (*b*) SEM image of a loosely packed mesh nanoES, showing the macroporous structure. (*c*) Confocal fluorescence micrographs of a synthetic cardiac patch. (II and III), Zoomed-in view of the upper and lower dashed regions in I, showing metal interconnects, the SU-8 scaffold (arrows in II) and electrospun PLGA fibres (arrows in III). (*d*) Epi-fluorescence micrograph of the surface of the cardiac patch. Green (Alexa Fluor 488): α-actin; blue (Hoechst 34580): cell nuclei. The position of the source–drain electrodes is outlined with dashed lines. (*e*) Conductance versus time traces

recorded from a single-nanowire FET before (black) and after (blue) applying noradrenaline. (*f*) Multiplex electrical recording of extracellular field potentials from four nanowire FETs in a mesh nanoES. Data are conductance versus time traces of a single spike recorded at each nanowire FET.