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Nanoelectronics Applications of Biosensors in Macromolecules Living Organisms Cells

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ABSTRACT

The interface between nanoscale electronic devices and biological systems enables interactions at length scales natural to biology, and thus should maximize communication between these two diverse yet complementary systems. Moreover, nanostructures and nano–structured substrates show enhanced coupling to artificial membranes, cells, and tissue. Such nano–bio interfaces offer better sensitivity and spatial resolution as compared to conventional planar structures. The exceptional synthetic control and flexible assembly of nan owires (NWs) provides powerful tools for fundamental studies and applications in life science, and opens up the potential of merging active transistors with cells such that the distinction between nonliving and living systems is blurred. In tissue engineering, nanotechnology can be applied to reproduce or repair damaged tissues. By using suitable nano material-based scaffolds and growth factors, artificially stimulated cell proliferation, in organ transplants or artificial implants therapy nano technology can be useful, which can lead to life extension. Nanoscale field effect transistors (nano FETs) as probes to study cellular systems, including the realization of nano FET comparable in size to biological nanostructures involved in communication using synthesized nano wires. We discuss future development in this research area, the unique challenges and opportunities, and the tremendous impact these nano FET based technologies might have in advancing biology and medical sciences.

INTRODUCTION

Metallic nanoparticles exhibit unique physical and chemical properties that make them valuable for environmental biotechnological applications [1]. Nano pharmaceuticals are an emerging field where the sizes of the drug particle or a therapeutic delivery system work at the nanoscale. Delivering the appropriate dose of a particular active agent to specific disease site still remains difficult in the pharmaceutical industry. Nano pharmaceuticals have enormous potential in addressing this failure of traditional therapeutics which offers site-specific targeting of active agents. Nano pharmaceuticals can reduce toxic systemic side effects thereby resulting in better patient compliance.

The growing interest in the use of super paramagnetic nanoparticles in environmental bioremediation may become of great importance in the development of new efficient technologies with the possibility to overcome the weakness of conventional nanoparticles. Overall, there is still much to do for the improvement of the nanotechnology systems before its application in bioremediation and continuous research is needed. Nanoelectronic-Biological Interfaces Enable:(1) Diagnostic devices for disease detection(2)General detection & kinetics platform(3)New tool for single-molecule detection/biophysics(4) Powerful devices for electronic and chemo/bio recording from cells, tissue & organs(5) Potential implants for highly functional & powerful prosthetics, as well as hybrid biomaterials enabling new opportunities.

Nevertheless, an understanding regarding the mechanism of nanoparticle formation is in its infancy. There is a need to control the size, shape and dispersity, which would be easier to manipulate if the exact mechanisms of the formation of nanoparticles are better known. High reproducibility of biomaterials is also a crucial point to be considered. Up to now, the formation of mainly Ag, Au, Cd and Pd nanoparticles has been studied, while knowledge of the synthesis of other metal nanoparticles is lacking. There is also a need to explore the microbial diversity to obtain novel microorganisms on nanoparticles synthesis and

to expand the range of oxide nanoparticles formed by microorganisms. Currently, neither the exact biochemical mechanism nor the genes and proteins involved are known. Modern techniques, like differential proteomics combined with genomics could be helpful in identifying the key proteins involved in the nanoparticle synthesis.

Biological processes are spatiotemporally mediated by the dynamic actions of molecular machineries which are often organized in nanoscale domains and exist in extremely low abundance. Therefore, there is a pressing need to develop biosensors that can offer high enough sensitivity to detect trace amount of target molecules or minute biological signals, and high enough spatial and temporal resolution to resolve the subtle biological activities. The emerging nanoelectronic biosensors provide unprecedented possibilities towards such ambition which is unattainable by the conventional bioanalytical methods.

The active conducting channel (sensing element) of a nanoelectronic biosensor is made of nanostructured semiconducting (NS-SC) materials whose current flow is completely or largely confined on the surface, and therefore, highly sensitive to minute electrical perturbations imposed by interacting biomolecules or electrogenic biological activities at or near its surface. Because of their nanoscale dimension, NS-SC materials can intimately interact with, thus highly and locally responsive to, similarly-sized biomolecules or biomolecular machineries. Compared to the conventional methodologies (e.g., biochemical, optical, biophysical), nanoelectronic biosensing may offer readily, rapid, and label-free detection with high or even ultimate (single-molecule) sensitivity,high temporal resolution (sub-millisecond), high spatial resolution (nanoscale), and easy integration with electronic readouts. Using lithographic micro fabrication or advanced nanofabrication techniques, nanoelectronic biosensors can be miniaturized for lab-on-a-chip developments and made in array format for parallel detection of multiple analytes.

A number of NS-SC materials can be employed for nanoelectronic biosensing, such as, silicon nanowires (SiNWs) [2], single-walled carbon nanotubes (SWCNTs) [3], and recently discovered graphene and its derivatives [4]. Nanoelectronic sensors provide a versatile platform for a dazzling spectrum of biosensing targets and purposes because the availability of different sensing materials enables various sensing mechanisms and device configurations, and diverse chemistry for biofunctionalization. Each NS-SC material possesses unique advantages and limitations while being applied to a particular application. The choice should be made based on balanced consideration on specific requirements on the performance, detection mechanisms to be utilized, ease of fabrication and recording, cost, and so on.

Nanoelectronic sensors have been used to detect the presence of biomolecules, cells, or microbes at extremely low concentration. Remarkably, SiNW transistors have demonstrated the ability to detect

single virus [5]; and single molecule detection of DNA-hybridization kinetics has been achieved by SWCNT transistors [6]. The promising and unique potential of nanoelectronic sensors for molecular diagnosis or studies are clearly evidenced by many studies in the past two decades.

Recently, several groups have attempted to interface nanoelectronic devices with live cells for detection of dynamic cellular activities, such as, secretion of signaling molecules and generation of bioelectrical signals [7,8]. In their landmark work published in 2006, Charles Lieber's group of Harvard University demonstrated the use of array of nanowire transistors for spatially resolved detection, stimulation or inhibition of nerve impulses (electrical spike or action potential produced by ion channel activities in a neuron) and their propagation along individual neurites (slender projection from the cell body of a neuron) [9]. In contrast to the invasive intracellular recording by patch-clamp technique, this nanowire approach provides noninvasive extracellular measurement; as compared to the extracellular recording by metal microelectrode, the nanowire approach offers unraveled spatial resolution. Later, the same group made a nano-transistor device at the tip of a bent silicon nanowire, and used it, as a novel alternative to patch-clamp yet with minimum invasiveness, to intracellularly record action potentials [10]. Very recently, Lieber group demonstrated a new type of SiNW transistor with a branched hollow nanotube to establish connection with the intracellular space for the recording of action potentials [11]. Nanoelectronic sensors can probe at single cell or sub cellular level (even nano-domains). With an array of them, signal distribution and

propagation in cell networks could be resolved, e.g., in a brain slice or heart tissue. It is envisaged that nanoelectronics-cell interface would be instrumental or revolutionary to the study of cell functions as well as to the discovery of drugs targeting on these functions [12].

Exciting progress has been made in interfacing nanoelectronics with biomolecules, cells, and tissues/organs. No doubt that the convergence of nanoelectronics and biological systems will bring profound impacts on fundamental biological studies, diagnosis, drug discovery, and development of human-machine interface. But currently, nanoelectronic sensing still remains as a scientist niche. To transform it to practical and widely accessible techniques, tremendous and interdisciplinary efforts are still needed, for examples, to develop facile and scalable approach to make these fine devices, and to understand better and optimally engineer the interaction/communication between the bio-system and the nano device.

High spatio-temporal resolution interfacing between electrical sensors and biological systems, from single live cells to tissues, is crucial for many areas, including fundamental biophysical studies as well as medical monitoring and intervention. Recording electrical signals from cells and tissues, such as action potentials in the nervous system, is central to areas ranging from fundamental electrophysiological studies to brain activity mapping and biomedical prosthetic applications [13]. Conventional methods based on glass micropipette electrodes have been widely used for intracellular action potential recording, and have shown excellent signal-to-noise ratio (SNR) and temporal resolution. However, these methods have constraints [13b, 13c, 14] that limit their applicability to simultaneous measurements from large numbers of cells with single-cell resolution. In addition, the typical micrometer size of these probes poses a challenge to recording from small subcellular structures and also results in invasiveness and toxicity to cells [13b, 14]. On the other hand, methods using micro fabricated metal electrodes and arrays (MEAs) have made possible large-scale multi-site recording, although the size of these electrodes remains micrometer scale in order to meet electrode/electrolyte interface impedance conditions necessary to achieve usable SNR. This size restriction precludes subcellular resolution needed for many important studies [13d, 13e, 15]. In the case of tissue level electrical measurements, implementing electronic sensors in three dimensions (3D) and the capabilities for monitoring cells throughout the 3D micro-environment of tissues is critical for functional neural activity mapping and understanding physicochemical changes relevant to living organisms [13a, 16]. Most work has, however, focused on coupling electronics to the surface of tissues or artificial tissue constructs, including recently reported studies which use flexible and/or stretchable planar devices that conform to tissue surfaces [17].

The nanometer size of nanoelectronic devices makes them advantageous for realizing high resolution and minimally invasive cellular- and subcellular-level interfaces between recording probes and biological systems, and making such interfaces on a large-scale and with high-density is of significant importance for mapping activity in the brain and other excitable biological systems [13a, 18]. Moreover, the bottom-up paradigm used for nanodevices fabrication that we have pioneered [19] enables the preparation of 3D free-standing, macroporous device arrays that can be utilized as the scaffolding for synthetic tissue constructs, and thus realize monitoring of cellular activity throughout 3D cellular Networks [20]. In this focus review, we will talk about the development of novel nanoscale devices for intracellular action potential recording and macroporous nanoelectronic scaffolds for 3D interfacing with synthetic tissue constructs. We will focus on field-effect transistor (FET) devices from semiconducting nanowires or nanotubes, where the active nanowire or nanotube channel serves as the voltage sensing element. There are several reasons that make this nanoscale FET approach attractive. First, there is no dependence on device/electrolyte interface impedance for an FET voltage sensor [21], which enables dramatic miniaturization of recording probes (compared to passive metallic electrodes); this miniaturization of the sensors facilitates both subcellular level resolution and high-density recording. Second, the structure, morphology, physical properties and corresponding functions of semiconducting nanowires and nanotubes can be well controlled by encoded synthesis, which makes them ideally suited for hierarchical design of devices [22].

Research at the interface between nanoscience and biology has the potential to produce breakthroughs in fundamental science and lead to revolutionary technologies [23,24].

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 [25–32] or components for integrated circuit [33,34]. These basic platforms, such as planar nanowire field effect transistors [33–37] or vertical nanowire arrays [26–29,31,32], have been used in bio molecular sensing [23,24], extracellular recording [23,24], drug delivery [38–40] and localized cellular imaging [41].

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 [42–45], and in so doing, open up completely new opportunities in science and technology. Examples of advanced platforms include recent intracellular field effect transistor probes [42,44–46] and nanoelectronics-innervated synthetic tissues [43].

FUNDAMENTALS OF NANO FET 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 [47]. 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 [42,48]. 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 [49–56].

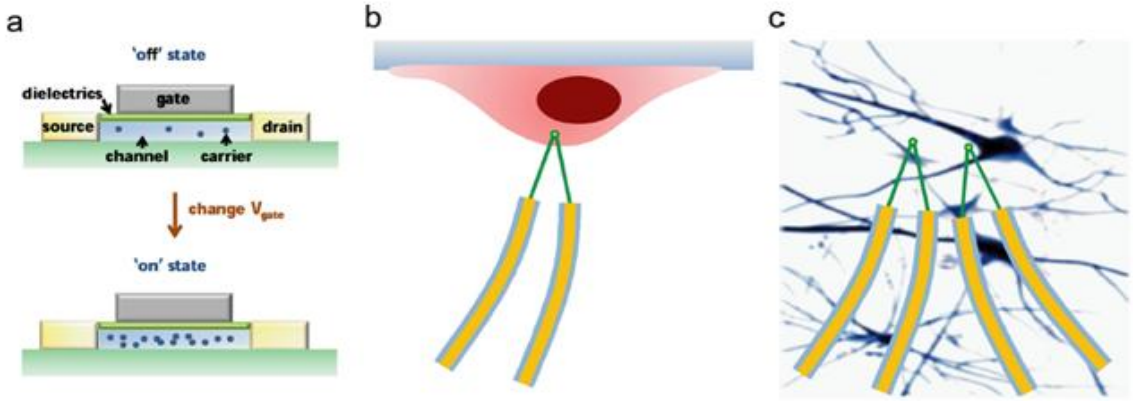


Figure1. *FET basics and electrical interfaces between nano FET and biological systems (a) Schematic of a planar FET device. In FET, current flows along a semiconductor path called the channel. At one end of the channel, there is an electrode called the source. At the other end of the channel, there 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 alarge 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 akinked 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.*

In integrated circuits, the basic device element is a multi-terminal FET that uses either electrons or holes as the charge carriers [57] (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 [58]. The Poisson equation [59] links such potentials directly to the ionic current sources and sinks that produce them. The Goldman-Hodgkin-Katz voltage equation [59] 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 c). The sensitivity of a FET or how well the transistor can receive and amplify the gate signal is usually defined as trans conductance (Gm) [23,24,57,60], which is inversely proportional to the dimension (L) of the active device [60]. 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 [42,44–46] and from the internal regions of synthetic tissues [43], 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 [44,45].

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 [61,62], 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 [63,64]. Other growth mechanisms, such as vapor-solid-solid (VSS) and vapor-solid (VS) [65], 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 [65–68], 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 [69–73] (Figure 2b) or radial directions [74–76] 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 [77]. 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 [78–82].

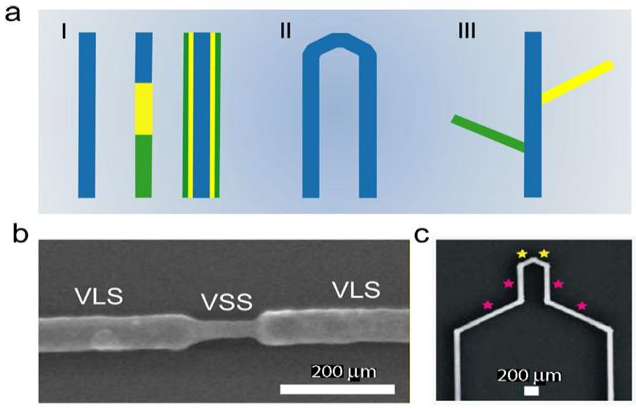


Figure2. *Semiconductor nanowire structural motifs for nanoFETs (a) Schematics of 1D (I), 2D (II) and 3D (III) motifs. 1D motif (I) can have uniform composition and doping (I, left) or axially (I, middle) or radially (I, right) modulated. Akinked nanowire with structurally coherent "kinks" introduced in a controlled manner during axial elongation represents an example of 2D motif (II). Hetero branched nanowires yield 3Dstructure (III) and the branch junction (e.g., blue/yellow segment junction) can be exploited for localized sensing. (b) An axial nanowire hetero structure 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.*

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) [42,83]. 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 [42].

A third basic motif involves the synthesis of branched or tree-like nanowire structures (Figure 2a, III) [84–86]. To this end, we reported a rational, multistep approach toward the general synthesis of 3D branched nanowire hetero structures [84].

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 nanowires well 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.

Multiplexed Extra cellular Electrical 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 multi electrode 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 [60,78,87], 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 [66,88], 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 bio molecules, including detection of single particles [24]. Second, bottom-up fabrication of nanoFETs yields devices that have nanoscale protrusions from the substrate surface [24,89]. 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 [33,36,90,91]. 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 [92–95]. 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~ 0.1μm2, is much smaller and can provide better spatial resolution of signals compared to MEA and planar FETs that are 102 to 105 times larger in active area [48]. Last, nanoFET detectors provide fast intrinsic response time which is critical for high temporal resolution recordings [82,96].

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 [97](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.

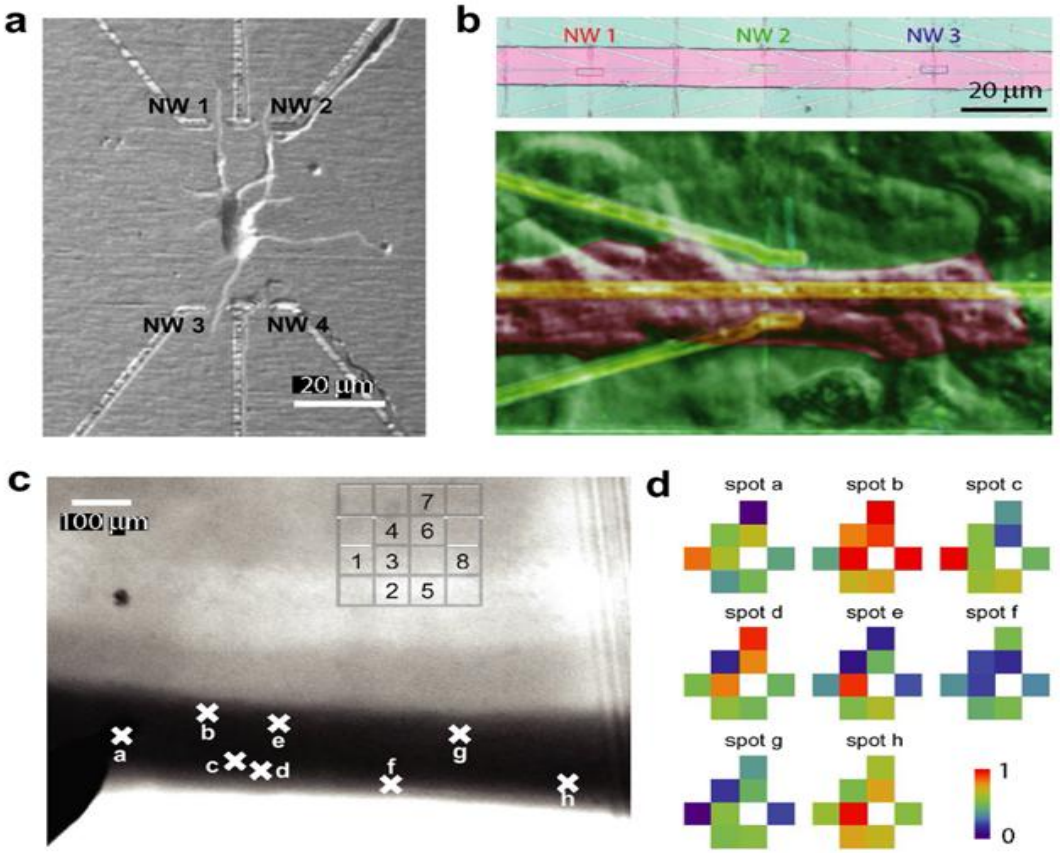


Figure3. *Multiplexed extracellular electrical recordings using nanoFETs (a) Optical image of a cortical neuron interfaced to three of the four functional nanoFETs in an array. (b) Upper panel, optical micrograph showing three nanoFET devices (NW1, NW2,and NW3) in a linear array, where pink indicates 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.*

Recording From Cardiomyocyte Monolayers

Multiplexed measurements using the nanoFET arrays interfaced with cultured embryonic chicken cardiomyocytes (Figure 3b) [98]. 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 in homogeneity 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.

Recording from Tissues and Organs

Finally, nanoFETs have been used to probe electrical activities from tissues and organs [48,99]. 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[48] (Figure 3c). Strong stimulation of all axons fibers in the LOT yielded similar response by nanoFETs 1–8with 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 perform cortex.

INTRACELLULAR ELECTRICAL RECORDING

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 [100]. 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 molecule 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 nano FETs into the cell and directly record intracellular electrical activities, which can provide much more detailed understanding of the inner workings of cells.

Although nanoFETs have been exploited for ultrasensitive detection of biological markers and high-resolution extracellular recording from cells [24], 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 [83,44–46]. 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 [101–104], and single-terminal glass or carbon microelectrodes as mentioned briefly in prior section [47,50](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 [102]. 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 [105–108].

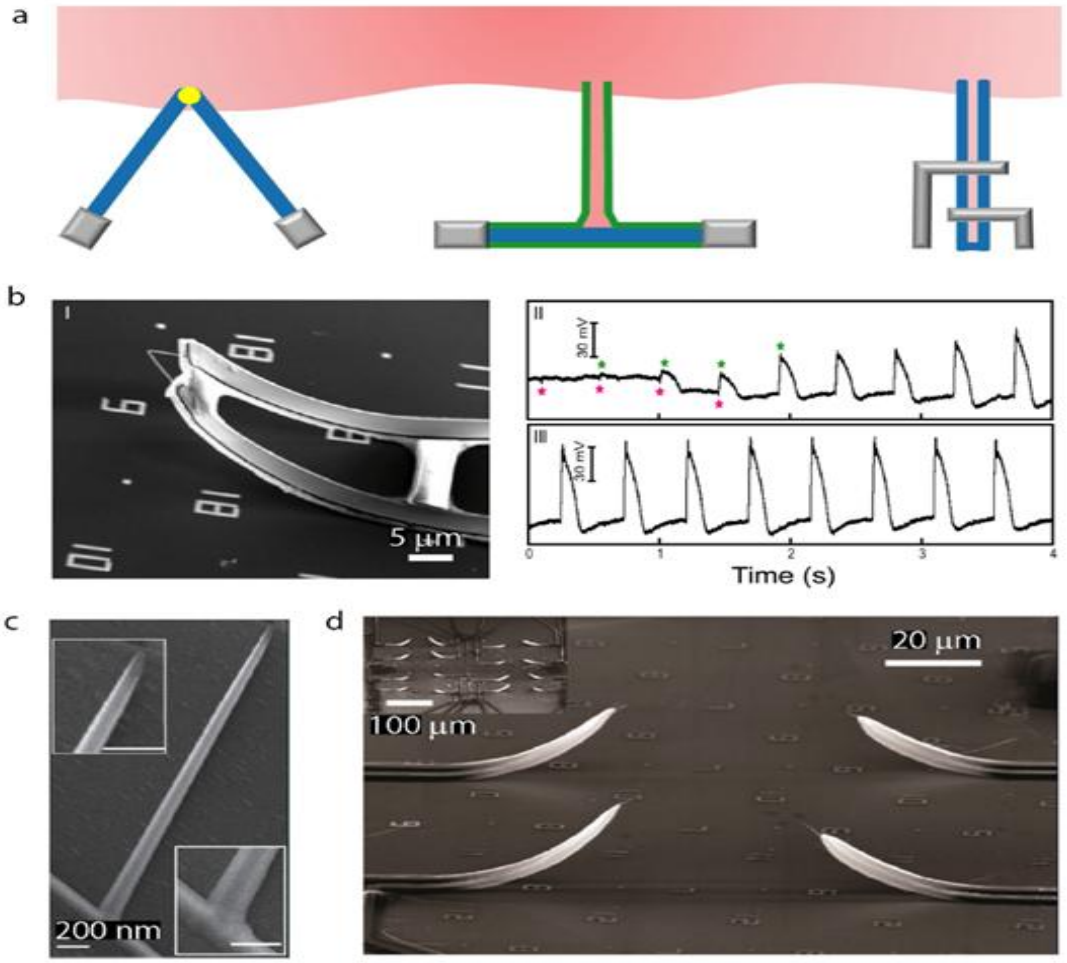


Figure4. *Intracellular electrical recordings using nanoFETs (a) Schematics of kinked nanoFET (left), BIT-FET (middle) and ANTT (right) probes. (b)SEM image of a kinked nanoFET probe (I) and its intracellular electrical recordings (II, III)from spontaneously beating cardiomyocytes. (c) SEM of a BIT-FET probe, insets highlight the tip and root parts of the hollow branch. (d) SEM image of ANTT probe array.*

NanoFETs can function in a sub–10-nm-size regime [109]. 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 [110–113].

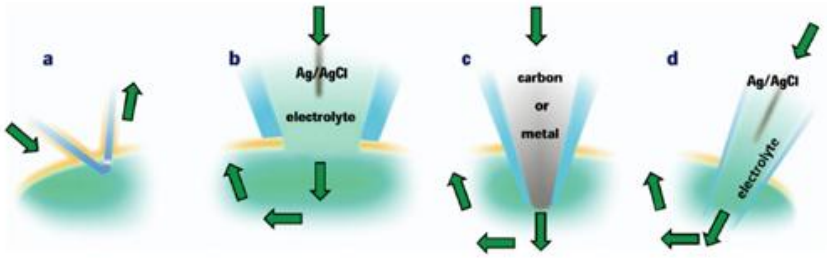
Moreover, when interfacing with cells, nanoFETs process input/output information without the need for direct exchange with cellular ions; thus, the issues of interfacial impedance and biochemical invasiveness to cells can be ignored or minimized (Figure 5).

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 [42, 44–46](Figure 4a) has now shown three solutions that open up these exciting opportunities.

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 [42](Figure 4a,b). This was achieved through control over cis-/trans- conformations and modulation doping during the silicon nanowire synthesis [42,83]. Subsequently, the freearms 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 nano FET 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 electro physiology.



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

Figure5. *A comparison between kinked nanoFET probe (a) and conventional intracellular tools (b–d).The green arrows in (a–d) indicate the current flows. Rs, series resistance; Rj, junction resistance; Rm, membrane resistance; Vm, intracellular potential; Cj, junction capacitance; Cm, membrane capacitance.*

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 SiO2 nanotube was synthetically integrated on top of a nano FET (BIT-FET) [44] (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 [45] (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).

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.

Nanoelectronics Innervated Synthetic Tissues

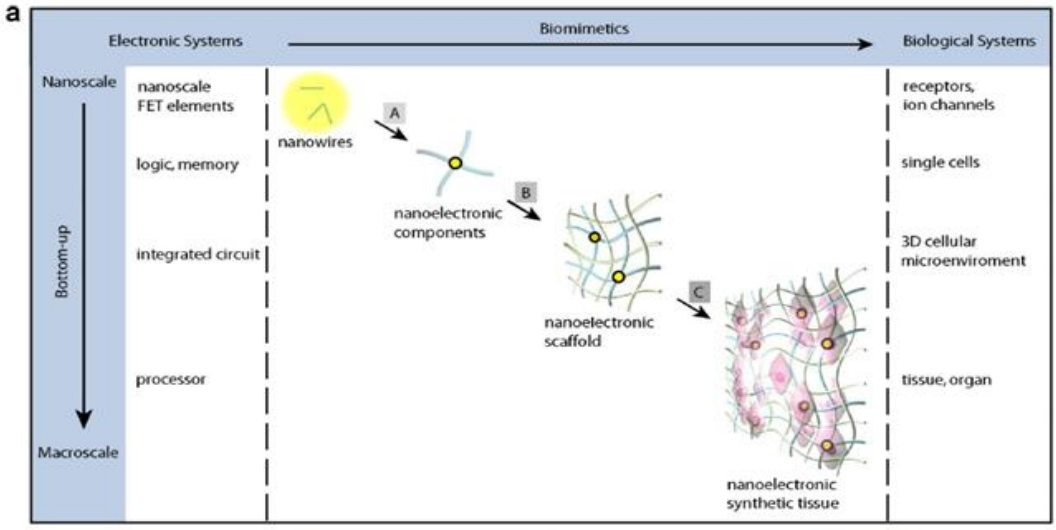
The development of synthetic 3D macroporous biomaterials as extracellular matrices (ECMs) represents a key area because (i) functionalized 3D biomaterials allow for studies of cell/tissue development in the presence of spatiotemporal biochemical stimulants [114,115], and (ii) the understanding of pharmacological response of cells within synthetic tissues [116–118] 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 [24,92–95,99,119], or implantable micro fabricated probes [120]. 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. 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 [24,99]. Merging electronics seamlessly throughout tissues (Figure 6a) had remained a major challenge. To address this challenge we recently set-forth the key constraints [43]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).

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 [43] (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). Metalelectrode 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.



b

	Targets	Approaches	Implementation Examples	Steps		
Biomimetics	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	

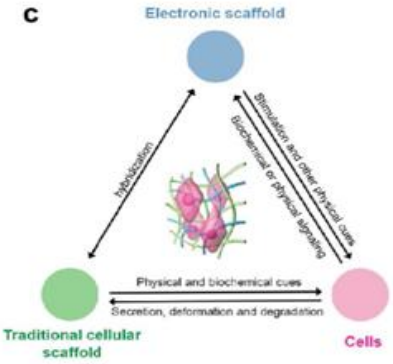


Figure6. *Integrating nanoelectronics with cells and tissue Conventional bulk electronics are distinct from biological systems in composition, structural hierarchy, mechanics and function. Their electrical coupling at the tissue/organ level is usually limited to the tissue surface, where only boundary 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 nano wire 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: nano wire 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.*

Designs and Preparation of Synthetic Tissues

We have designed two types of 3D macroporous nanoES (reticular- and mesh- nanoES) to mimic the structure of natural tissue scaffolds (Figure 7) [43]. 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, cardio myocytes and smooth muscle cells (Figure 7c,d). Significantly, we have demonstrated multiplexed electrical recordings of extracellular field potentials from 3Dnanoelectronic 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 nano electronic devices for simultaneous monitoring of pH inside and outside an engineered tubular vascular construct that was developed from the nano electronic scaffold, suggesting the potential of a multifunctional prosthetics.

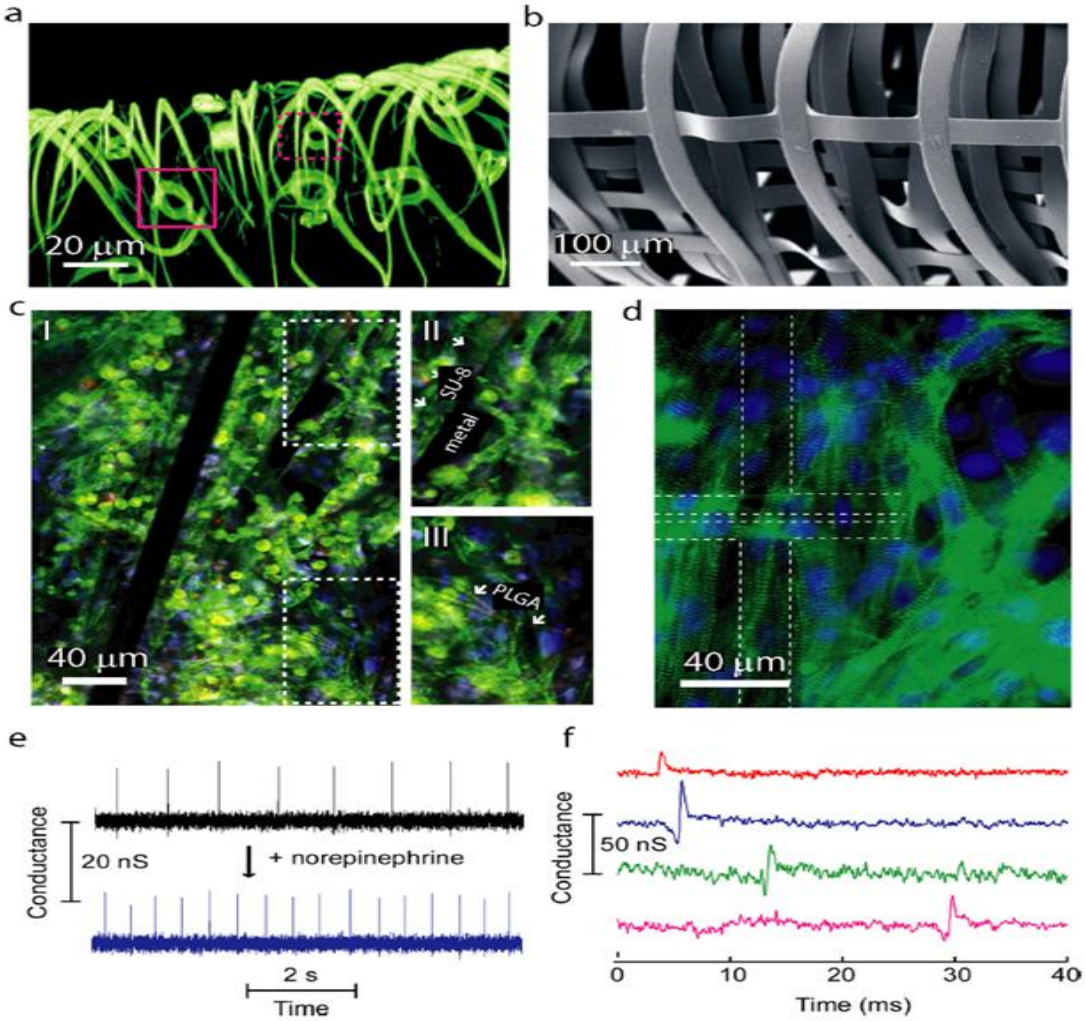


Figure7. *NanoES exhibited robust electronic properties recorded from a single-nanowire FET before (black) and after (blue) applying noradrenaline. (f) Multiplex electrical recording of extracellular field potentials from four nanowire FETs in a mesh nanoES. Data are conductance versus time traces of a single spike recorded at each nanowire FET.*

CHALLENGES AND PROMISES

The 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 [121] by exploiting the diverse nanowire building blocks available from designed synthesis. Cell or tissue interactions with nanoES could be finetuned by modification with cell growth determinants [116]. 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 nano ES 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.

APPLICATION AREAS OF BIOSENSORS AND –ASSAYS

Biosensor: A device that uses specific biochemical reactions mediated by isolated enzymes, immune systems, tissues, organelles or whole cells to detect chemical compounds, usually by electrical, thermal or optical signals.

Bioassay: A bioassay is a procedure for determining the concentration, purity, or biological activity of a substance by measuring the biological response that it produces compared to a standard

- Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)
- Hormones (steroids)
- Proteins (polypeptides)
- Immune globulins IgG, IgM, IgA, IgD, IgE ⇒ immunoassays (antibody-antigen reaction) Determination of
- A single analyt ⇒ Single analyt assays
- Several analyts ⇒ Multi analyt assays.

Biosensors can be applied in detection of:

- Glucose in blood
- Cancer markers in blood
- Penicillin in fungi bioreactors
- Urea in urine

PRINCIPLE OF OPERATION

The techniques of operation is shown in Figure 8,where as, the analyte, receptor and transducer are as following:

Analyte

- The substances to be measured
- Small molecules: Sugars, urea, cholesterol, glutamic acid, phosphate,
- Macro molecules: Nucleic acids (DNA, RNA), poly peptides (protein, antibody, enzyme).

Receptor

- A sensing element that responds to the substances being measured
- The interaction must be highly selective ⇒ Enzyme, Antibody, Nucleic acids, Cells.

Transducer

A device that converts the physical or chemical changes due to analyte receptor reaction to another form of physical signal (in general, electronic signals) whose magnitude is proportional to the amount of the analyte

Electrochemical detection Potentiometric, Voltammetric, Conductimetric

Optical detection Fluorescence, Absorbance, Light scattering, Refractive index

Electrical detection Field effect transistor (FET)

Mechanical, Thermal, Piezoelectric, Surface acoustic waves, Magnetical, ….

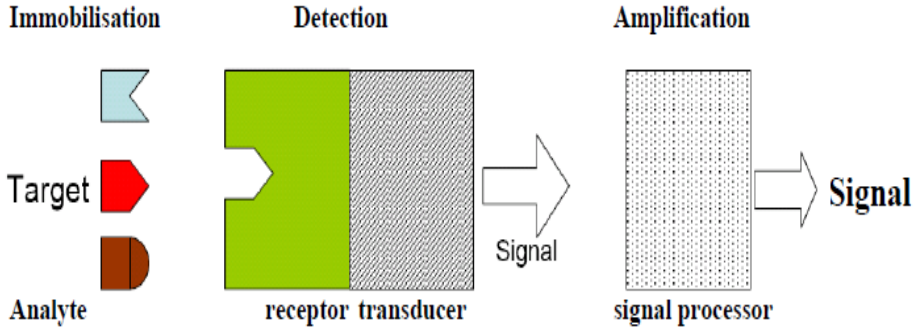


Figure8. *Principle of biosensors operation*

Performance Factors

Sensitivity

- Minimum amount of analyte that are able to be detected above the background
- Units: Concentration, number of analyte, density, weight

Specificity/Selectivity

- The ability to discriminate between substrates. This is function of biological component, principle, although sometimes the operation of the transducer contributes to selectivity
- Molecular recognition
- Separation scheme
- Signal overlap

Speed/Response Time

- Sample preparation + Biological/Chemical reaction + Signal Processing
- Bench process: hours to weeks

- Chip process: minutes to hours
- Ultra-high temporal resolution, 10 ns, for real-time measurement of molecular kinetics

Moreover: Accuracy, Simplicity, Cost, Lift time

Examples

Conversion of Bio Molecules by an Enzyme Bound to a Surface (E.G. Polyaniline)

As given in Table 1. , and Figure 9

Table1. *Examples of conversion of bio molecules by an enzyme*

Enzyme	Reaction
GOD	glucose + O_2 → gluconic acid + H_2O_2
Urease	$(NH_2)_2CO$ + H_2O → $2NH_3$ + CO_2
Catalase	H_2O_2→ H_2O + O_2
Trypsin	polypeptide → amino acids
Amylase	starch → glucose
Uricase	uric acid + H_2O → NH_3 + CO_2

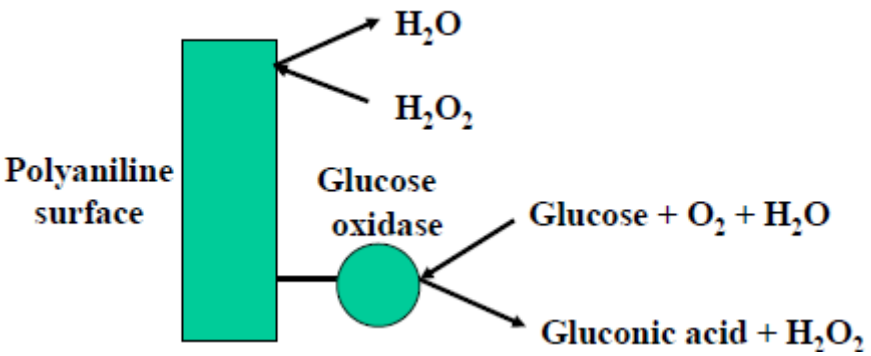


Figure9. *Conversion of bio molecules by an enzyme.*

Biosensing of Macromolecules

Figure 10 represents the steps of the biosensing process.

Steps: (1) Immobilization of primary probes

(2) Mixing/incubation of the mixtures

(3) Washing the non-specific bindings

(4) Signal transduction

Markers/Report molecules

- Antibody-antigen binding Ab + Ag → Ab-Ag
- Enzymes
- Fluorescence tags: nanoparticles and fluorescent dyes
- Radioactive tags

Molecular recognition

- Watson-Crick base pairing. ATTGGC (target) + TAACCG (probe) → ATTGGC TAACCG

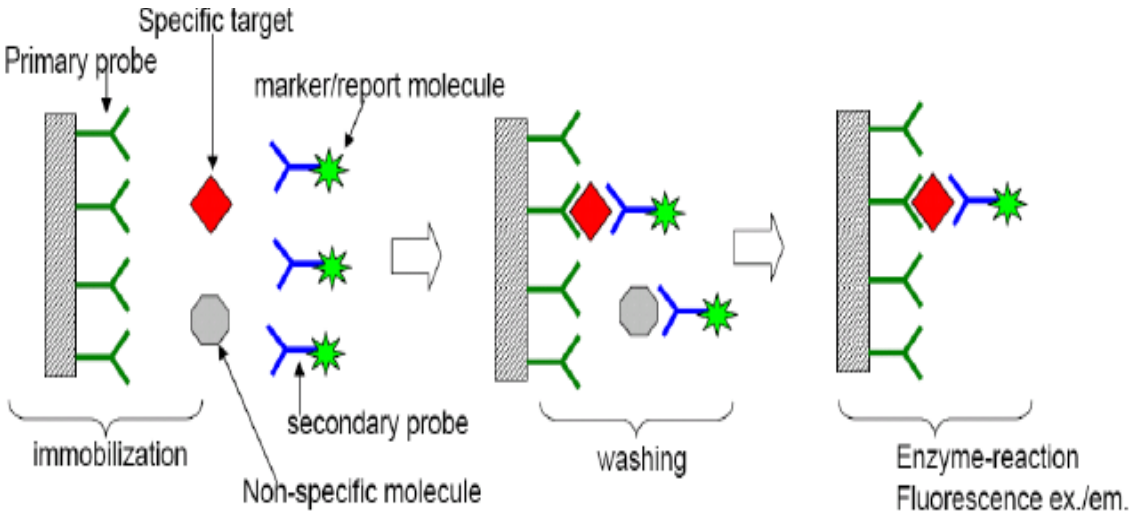


Figure10. *Biosensing of macromolecules*

Nanowire Nanosensor

Binding of chemical or biological species to the surface of a nanowire will result in depletion or accumulation of carriers. The change in carrier concentration due to binding can be directly monitored by measuring the nanowire conductance (Figure 11).

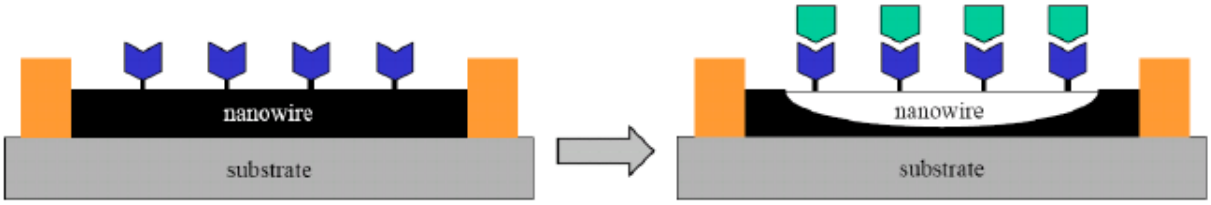


Figure11. *nanowire conductance*

A solid state FET, whose conductance is modulated by an applied gate, is transformed into a nanosensor by modifying the silicon oxide surface. The conductance of modified Si-NWs increases stepwise with discrete changes in pH from 2 to 9. Changes in the surface charge can chemically gate the Si-NW (Figure 12).

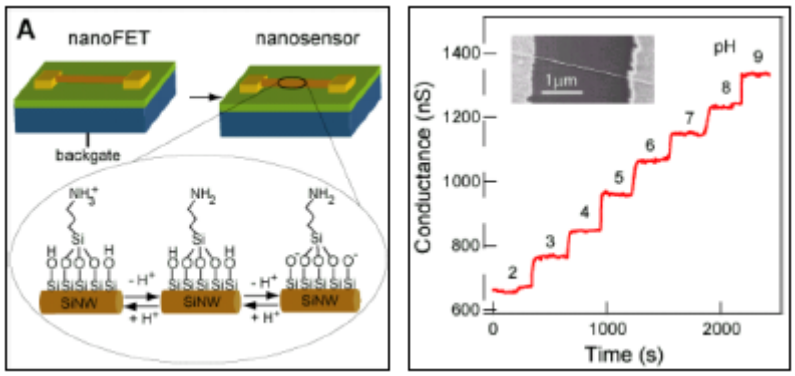


Figure12. *The conductance of modified Si-NWs*

Multi Analyte Nanoparticle Release Bioassay

As shown in the Figure 13.

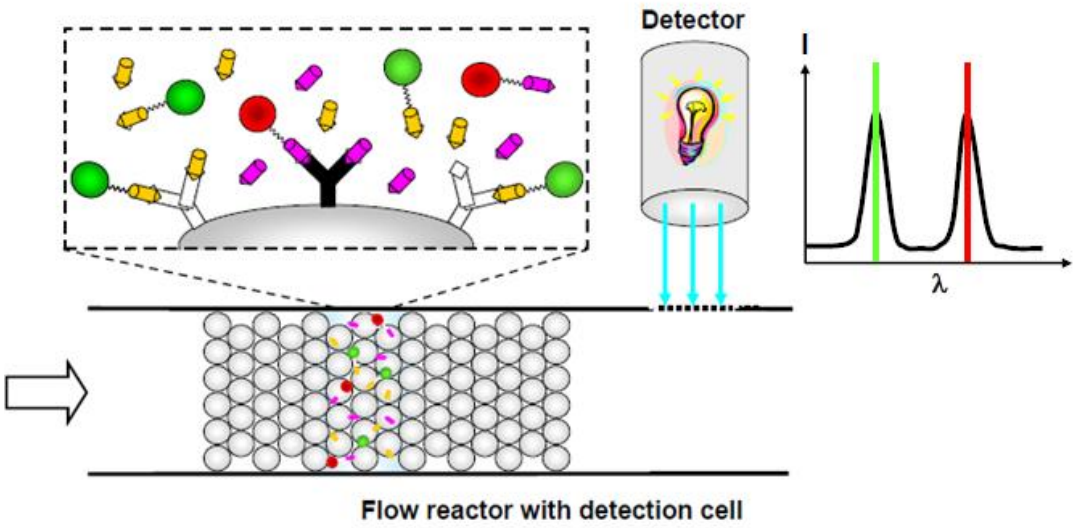


Figure13. *Multi analyte nanoparticle release bioassay*

CONCLUSIONS

Nano materials have increased surface area and nano scale effects, hence used as a promising tool for the advancement of drug and gene delivery, biomedical imaging, diagnostic biosensors and biobnanoelectronics. Nano materials (NWs and NPs) have unique physicochemical and biological properties as compared to their larger counterparts. The

properties of nano materials can greatly influence their interactions with bio molecules and cells, due to their peculiar size, shape, chemical composition, surface structure, charge, solubility and agglomeration. For example, nano particles can be used to produce exceptional images of tumor sites; single-walled carbon nanotubes, have been used as high-efficiency delivery transporters for biomolecules into cells. There is a very bright future to nano technology, by its merging with other technologies and the subsequent emergence of complex and innovative hybrid technologies. Biology-based technologies are intertwined with nanotechnology-nanotechnology is already used to manipulate genetic material, and nano materials are already being built using biological components. The ability of nanotechnology to engineer matter at the smallest scale is revolutionizing areas such as information technology cognitive science and biotechnology and is leading to new and interlinking these and other fields. By further research in nanotechnology, it can be useful for every aspect of human life. Medicine, regenerative medicine, stem cell research and nutraceuticals are among the leading sectors that will be modified by nanotechnology innovations. Finally, the nanoscale precision and the detailed investigation that these nanoelectronics and nanometrology techniques offer, give them an enormous potential for even more advanced applications for the improvement of the quality of research and of the everyday life.

Conflict of Interest

The author declared there is no conflict of interest.

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