Biosensors for Cell Analysis

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Abstract

Biosensors first appeared several decades ago to address the need for monitoring physiological parameters such as oxygen or glucose in biological fluids such as blood. More recently, a new wave of biosensors has emerged in order to provide more nuanced and granular information about the composition and function of living cells. Such biosensors exist at the confluence of technology and medicine and often strive to connect cell phenotype or function to physiological or pathophysiological processes. Our review aims to describe some of the key technological aspects of biosensors being developed for cell analysis. The technological aspects covered in our review include biorecognition elements used for biosensor construction, methods for integrating cells with biosensors, approaches to single-cell analysis, and the use of nanostructured biosensors for cell analysis. Our hope is that the spectrum of possibilities for cell analysis described in this review may pique the interest of biomedical scientists and engineers and may spur new collaborations in the area of using biosensors for cell analysis.

Contents

1. INTRODUCTION

A biosensor is a device that converts biological phenomenon into a recordable signal. The history of modern biosensors may be traced to the early 1960s, when Leland C. Clark detected glucose in an aqueous solution (1, 2). During the past two to three decades, the field of biosensors has expanded exponentially, spurred on by the demand for new and improved approaches for diagnosing disease, controlling biotechnological processes, improving food safety, and detecting biological warfare. A typical biosensor is divided into three parts: (*a*) a biorecognition element that ensures the specificity of the interaction with the target analytes, (*b*) a transducer that converts the interaction between the biorecognition element and the target analyte into a detectable signal, and (*c*) an interfacial or biopackaging layer that mediates the interaction between the device and the sample. This article focuses on the biorecognition elements and biopackaging components of biosensors. As for target analytes, we review the detection of biomolecules excreted or processed internally by cells.

2. ANTIBODY-BASED BIOSENSORS FOR CELL ANALYSIS

The most widely used antibody-based assay is the enzyme-linked immunosorbent assay (ELISA). A typical ELISA involves surfaces functionalized with antibodies, and soluble secondary antibodies labeled with enzymes or fluorophores, that reveal the presence of captured antigen molecules. The ELISA concept has been leveraged to study cell secretions. One commercial product, the enzyme-linked immunospot (ELISpot), has become a mainstay of immunological techniques for monitoring the cytokine responses of immune cells (3). A variant of ELISpot, called T-Spot.*TB* (Oxford Immunotec, Marlborough, Massachusetts), which detects the release of interferon

(IFN)-γ, has become a valuable diagnostic tool for tuberculosis (4). In a typical ELISpot assay, the cells of interest are seeded together with antigens onto a polyvinylidene fluoride membrane coated with monoclonal or polyclonal antibodies. Cytokine molecules secreted by the activated cells are captured locally by the antibodies surrounding the cells, and are visualized by labeling with secondary enzyme-linked antibodies. Each spot represents one cytokine-secreting cell.

2.1. Miniaturizing Antibody-Based Assays

Micro- and nanotechnologies are being employed to make antibody-based assays simpler, smaller, more sensitive, and more informative. One strategy is to create micropatterned surfaces that juxtapose sites for the attachment of the desired cells and the detection of the cytokines of interest. In one example, CD4 and CD8 T lymphocytes from human blood were captured on anti-CD4 or anti-CD8 spots, and secreted cytokines were detected on the neighboring interleukin (IL)-2, tumor necrosis factor (TNF)-α, and IFN-γ antibody spots (**Figure 1*a–c***) (5). Subsequent iterations of this technology have involved the use of lens-free imaging to enable rapid quantification of cell numbers and cytokine signals (6), and the use of reconfigurable microfluidic devices to enhance the detection of secreted cytokines (7). Because it is important to integrate different biorecognition elements into a small footprint, Zheng et al. (8) have developed microfluidic devices with poly(dimethylsiloxane) (PDMS) buttons that can be actuated for in-channel patterning of capture probes. The Heath group (9) proposed a strategy for spatially stratifying biorecognition elements, such as DNA probes or antibodies, in microfluidic devices, which they termed the DNA-encoded antibody library (or DEAL) technique. In this approach, antibodies against cell-surface markers and intra- or extracellular protein targets were labeled with distinct DNA oligomers, and self-assembled to designated locations in a preprinted DNA array, which allowed for simultaneous detection of single-stranded DNA, proteins, and cell populations in the same chamber.

2.2. Amplifying Antibody-Based Detection

During the past decades, significant efforts have been made to enhance the sensitivity of cell analysis. One example of signal amplification is the in situ proximity ligation assay, developed by Soderberg et al. in 2006 (10). In this assay, oligonucleotides are employed as proximity probes and are covalently linked to two antibodies that recognize different proteins in the same complex. Upon antibody binding to epitopes, the two oligonucleotide probes are in close proximity and can be used as a template for generating complementary oligonucleotides in a process called rolling-circle amplification. Similar to polymerase chain reaction (PCR), several cycles of oligonucleotide replication can be used to amplify the signal associated with antibody binding. Replicating DNA is detected using intercalating dyes. Using this platform, Soderberg et al. investigated the phosphorylation of platelet-derived growth factor receptor-β in fixed cells, as well as in human scar tissue (11). A benefit of the in situ proximity ligation assay is that the signal arises from specific sites in the cell, thus providing new information about subcellular compartments where interactions occur. Thus, the interaction between the transcription factor c-Myc and Max, another oncogenic transcription factor, was characterized at single-molecule resolution (10).

2.3. Label-Free Detection of Cell Secretions

Several approaches employ antibody-modified devices for label-free detection of cell secretions. One approach employs surface plasmon resonance (SPR). This technique monitors the interaction of light with a thin layer of conductive material, typically gold. A certain angle of incidence of the

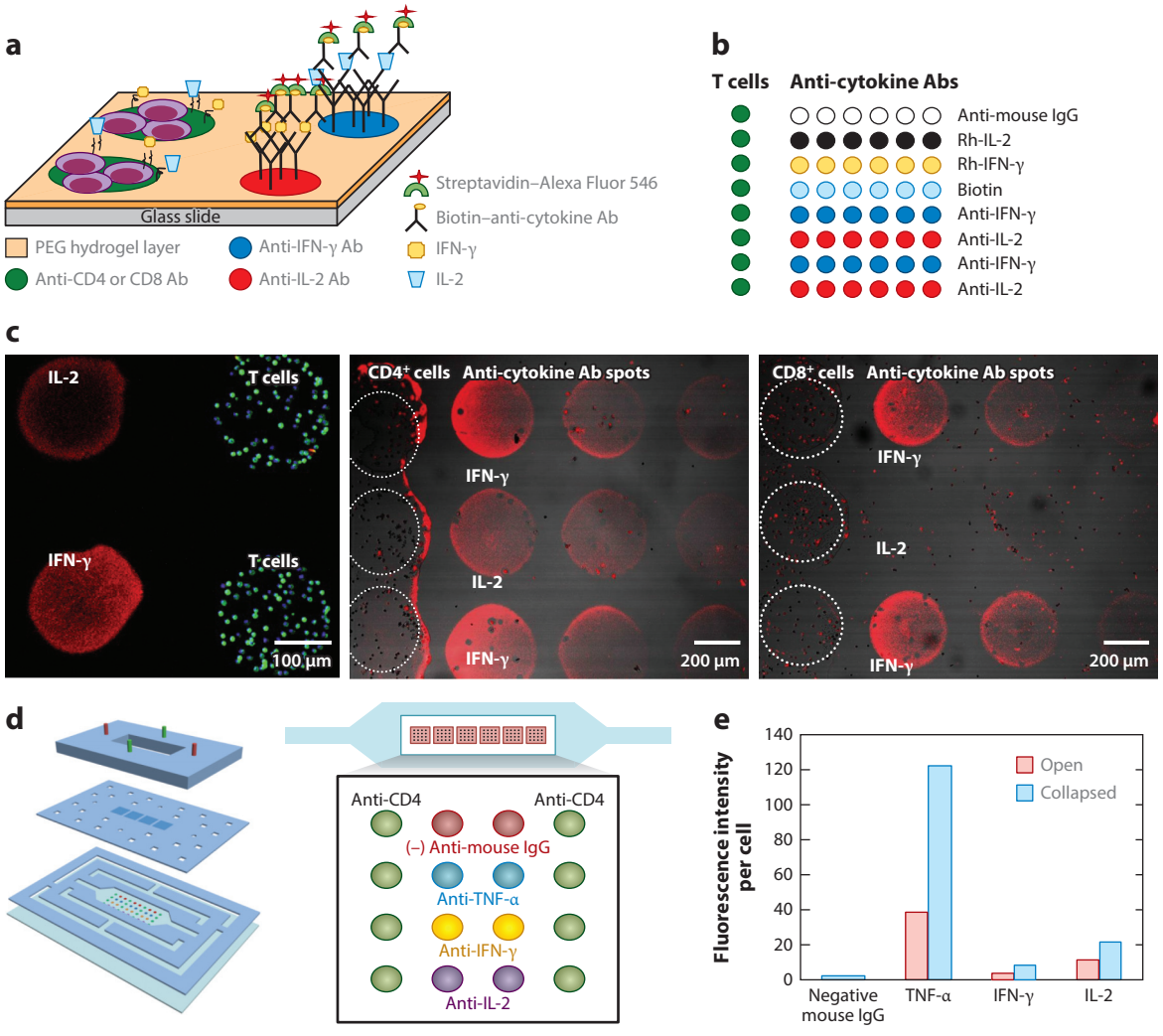

Figure 1

Miniaturized antibody-based assays. (*a*,*b*) Schematic illustration of microarrays used to detect cytokines secreted by T cells (Alexa Fluor 546, Life Technologies, Grand Island, New York) (5). (*c*) Detection of secreted cytokines from different subsets of T cells using antibody (Ab) microarrays (5). (*d*) Scheme showing the principle of using a collapsible roof device to enhance sensitivity (7). (*e*) Graph showing the relative fluorescence intensity of the roof device in the collapsed and open configurations (7). Abbreviations: IFN, interferon; IgG, immunoglobulin; IL, interleukin; PEG, poly(ethylene glycol); TNF, tumor necrosis factor.

light results in the coupling of an evanescent wave with a surface plasmon wave within the metal layer, and leads to a minimum intensity of reflected light. This minimum, or the angle of incidence at which the minimum occurs, is a sensitive function of molecules interacting with surfaces. Functionalizing the surface of the SPR instrument with capture probes allows the detection of target analytes in the absence of labels (12). Stybayeva et al. (13) reported a system that combines selective cell separation from blood with subsequent label-free detection of IFN-γ using SPR. However, more cost-effective alternatives to the commercial prism-based SPR system have been developed to monitor alterations in the local refractive index induced by surface–molecular

interaction. Gao et al. (14) combined a Mach–Zehnder interferometer with a microfluidic system and developed an ultrasensitive biosensor that integrates nanoslit-based plasmonic architecture with sensitive interferometric techniques. The sensitivity of the refractive index was enhanced [>3500 nm/RIU (refractive index unit)] (14). Afterward, the same group proposed a significantly simpler platform composed of arrays of circular aperture–groove nanostructures and collinear transmission geometry; they found that this maintained a favorable sensor resolution when compared with prism-based SPR sensors (15).

Another class of label-free biosensors relies on microring resonators, devices originally developed for signal processing in the telecommunications industry. The sensing modality is based on interactions between light and matter: the light propagating through the microrings results in a decaying evanescent field that extends beyond the surface and allows for efficient coupling to the ring at a certain wavelength (λ). As a result, the binding of targets to receptors on the ring's surface leads to a shift in the resonant wavelength, thus generating a detectable signal. Wang et al. (16) have applied this technology to detect physical alterations on the cellular level, such as cell adhesion and growth. On the molecular level, Luchansky & Bailey (17) demonstrated direct cytokine detection in 5 minutes with a sensitivity down to 100 pM. Moreover, they have used microring sensors for cell analysis by probing the temporal secretion patterns of primary human T cell subsets (Th0, Th1, and Th2) differentiated from naive CD4+ T cells, and proved the existence of unique cytokine signatures in each subset of T helper cells at the early stage of differentiation.

3. NUCLEIC ACID-BASED BIOSENSORS FOR CELL ANALYSIS

In addition to antibodies, nucleic acids, including DNA and RNA, are also widely used to detect and quantify biomolecules. Nucleic acid probes are useful not only for detecting other nucleic acids involved in gene transcription or regulation [e.g., messenger RNA (mRNA)] but also offer exciting opportunities for detecting proteins either secreted or presented on cellular surfaces. Below we discuss nucleic acid-based biosensors, loosely dividing them into those monitoring intracellular and extracellular spaces.

3.1. Detecting DNA or RNA Inside Cells

DNA or RNA is detected by hybridizing single-stranded DNA or RNA with its complementary strand through base pairing. The standard detection approach involves amplifying DNA via PCR, and then using gel electrophoresis to detect DNA fragments. Progress in the technologies used to detect DNA or RNA has included the development of new, labeled and label-free assays.

Most labeled assays for DNA or RNA use hybridization probes. One particular format, the molecular beacon, has garnered significant interest. A molecular beacon is usually a hairpin structure that contains a target complementary sequence in its loop domain, with a fluorescence resonance energy transfer (FRET) pair placed in proximity to both ends of the stem. When the beacon molecules unfold, the fluorescence signal turns on (18). Notably, this molecular beacon-based signal-on method has extraordinary advantages for studying mRNA in living cells. Due to the effects of FRET, these probes become fluorescent only when recognition events take place, thus allowing imaging of the processes occurring inside live cells (19). The concept of intracellular sensors was advanced further through the development of nanoflare reagents—that is, gold nanoparticles conjugated with an oligonucleotide hairpin. In this type of probe, the proximity of the fluorophore to gold causes quenching, and displacement of the fluorophore upon binding of the target mRNA causes fluorescence to turn on (20). Nanoparticles are taken up by cells with relative ease, serve as carriers of beacons, and obviate the need for conventional transfection reagents. To account for

cell-to-cell variation, Prigodich et al. (21) established a multiplexed nanoflare platform that allows the simultaneous detection of two distinct mRNA molecules in one living cell. Thus, with one of the mRNA targets serving as an internal control, the relative mRNA level can be determined (21).

Nucleic acids have unique physical and chemical properties that may be used for detection. It is possible to monitor the accumulation of negative charges on their surfaces upon hybridization. DNA is intrinsically negatively charged due to the phosphate ions in the backbone. Semiconductor devices, typically field-effect transistors (FETs), are sensitive to variations in charges at the surface–electrolyte interface. By immobilizing probes onto the surface of a transistor gate, the variation in charge induced by hybridized complementary oligonucleotides can be electrically detected and quantified (22). In an evolution from planar FET, a more robust semiconductor platform—a nanowire-based transistor—has attracted significant interest. The high surface-to-volume ratio of nanowires pushes the detection limit of DNA down to the femtomolar range from the micromolar range.

3.2. Aptamer-Based Biosensors

After the discovery of aptamers, the detection scope of nucleic acid-based biosensors expanded from oligonucleotides to other species, including proteins and even whole cells. Aptamers, first reported in 1990, are single-stranded DNA or RNA molecules that are able to bind to specific targets with high affinity (23). Similar to antibodies, aptamers can also serve as biorecognition elements in biosensors; however, a number of unique features make aptamers superior to antibodies for this application. First, the selection and amplification process for aptamers, usually referred to as the systematic evolution of ligands by exponential enrichment (known as SELEX), is an in vitro process that eliminates the need for cell lines or animals. Second, the chemical structure of aptamers is simpler and easier to modify than that of antibodies. Third, the stability of aptamers is significantly higher than that of antibodies and, thus, they are suitable for use in extreme conditions and resource-limited settings.

The majority of aptamer-based biosensors are presumed to function by changing conformation upon analyte binding. Various signal-transduction methods have been reported in recent years, with particular emphasis being placed on electrochemical methods. The Plaxco group (24) described immobilizing aptamer molecules on gold electrodes, labeling these DNA molecules with the redox reporter methylene blue, and using these electrodes to sense thrombin in blood serum. Aptamer architecture is such that the molecules undergo conformational changes as result of analyte binding and, thus, change the position of the redox reporter with respect to the electrode's surface. A signal-on aptasensor is one in which moving the redox reporter closer to the electrode's surface produces an on signal (24); architectures where the signal decreases upon analyte binding are known as signal-off aptasensors (25). Because the signal is generated directly upon analyte binding, without the need for washing or labeling steps, aptamer-based biosensors are well suited for the dynamic monitoring of analytes. Liu et al. (26, 27) used aptamer-based biosensors to monitor the release of the inflammatory cytokines IFN-γ and TNF-α from immune cells cultured inside microfluidic devices. The concept of miniature aptasensors integrated into cells was further enhanced by the introduction of reconfigurable microfluidic devices (**Figure 2*a*,*b***) that could be used for on-chip regeneration of aptasensors (28, 29) or for controlling communication between cells (30). The on-chip regeneration of aptasensors is particularly important because it allows the lifetime of the affinity biosensor to be extended, and may, in the future, enable multiday cell-detection experiments. Another exciting application of aptasensors is using them for in vivo monitoring. In this case, the possibility of monitoring dynamic changes in signals that is afforded by aptasensors is particularly attractive. Ferguson et al. (31) integrated signal-on

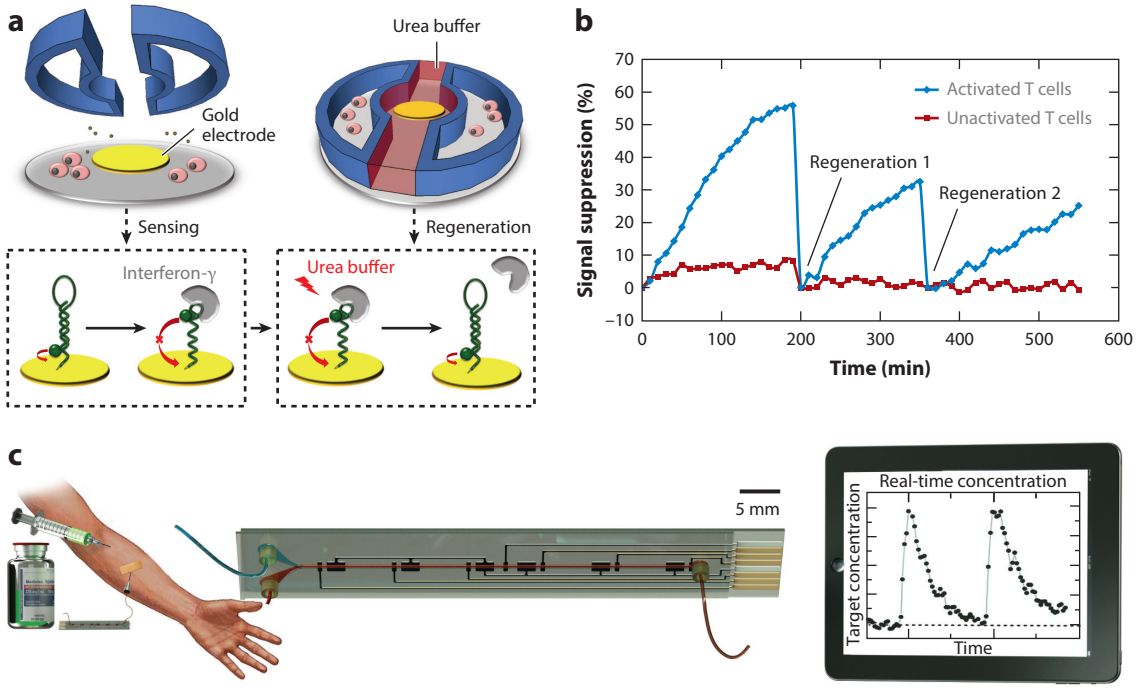

Figure 2

Aptasensors used in vitro and in vivo for real-time sensing. (*a*) Scheme representing the sensing and regeneration of aptamer-based sensors used to detect cytokines from leukocytes (28). (*b*) Real-time monitoring of cytokine production from cells for 9 h with two regeneration cycles (28). (*c*) Aptasensor connected directly to the bloodstream, and a typical monitoring curve of drug pharmacokinetics derived from an aptasensor (31).

aptasensors into the bloodstream of rats and humans directly, and realized in vivo continuous monitoring of doxorubicin (an anticancer drug) and kanamycin (an antibiotic) (**Figure 2*c***).

In addition to electrochemical sensing, there is considerable interest in developing fluorescence-based aptasensors (32–34). Such beacons are exciting for use as reagents for cell analysis in vitro (35) or in vivo (36).

Progress in nanotechnology has introduced novel applications for using aptamers in sensing. Cho et al. (37) have electrostatically bound vascular endothelial growth factor-165 ($VEGF_{165}$) aptamers to gold nanoparticle surfaces and proposed using a single-step aptasensor to detect cancer biomarkers. In this platform they make use of two features: the interaction between metal and fluorophore, termed surface-enhanced fluorescence, as well as the enhancement of incident light due to local SPR. Interactions among metal and fluorophore-labeled aptamers increased the radioactive fluorescent decay rate, which is avoided when aptamers bind to $VEGF_{165}$ and detach from the gold nanoparticle surface, allowing for a readout of the target concentration. Using a similar approach with nanoparticles, Zheng et al. (38) conjugated aptamers to gold nanospheres and hybridized aptamer-recognition elements using nanoflare techniques to achieve sensing for intracellular molecules, such as adenosine triphosphate (ATP), the most important molecule regulating cellular metabolism. Hu et al. (39) reported using another aptamer–nanomaterial complex (known as DNA nanoflowers) to perform cell imaging and drug-delivery tracing in cells. These

complexes emit multiple fluorescence signals upon single-wavelength excitation due to the three different dye molecules incorporated into the DNA matrix.

4. ENZYME-BASED BIOSENSORS FOR CELL ANALYSIS

Enzymes are frequently used as biorecognition elements in biosensors. In fact, enzyme-based biosensors were the earliest biosensors in the modern era. Compared with affinity-based biosensors, enzymatic sensors usually have lower detection limits because enzymes are not consumed but are continuously reused between catalyzing reaction cycles. The most widely used enzyme-based biosensor is the glucose meter, which was commercialized for diabetes management. In recent years, efforts have been made to reduce the impact of confounding factors on glucose sensors. Strategies such as using permselective membranes or self-referencing electrodes have been used and are well summarized in the literature (1). In this review, we focus on recent advances in the use of novel materials for enzyme incorporation, and the direct detection of enzymes from living cells.

One strategy is to integrate enzymes with carbon nanotubes (CNTs). The 3D structure of CNTs significantly increases the amount of enzyme that can be absorbed; also, catalytic activity has been proved to be well preserved in the CNT structure (40). More recently, graphene, a structural element in CNTs, has also proved to be an excellent scaffold for enzyme immobilization due to its high surface area, remarkable conductivity, and mechanical strength. Wu et al. (41) assembled glucose oxidase on graphene and reported effective direct electron transfer on the graphene surface, which exhibited a wide linear range (0.1–10 mM) and a low detection limit (10 ± 2 μM). Derivatives of graphene have also been applied to sensors. One example is the graphene oxide nanosheet sensor developed by Liu et al. (42). In this platform, amines of glucose oxidase were covalently attached to carboxyl acid groups in graphene oxide sheets, resulting in a biosensor with excellent reproducibility and operational and storage stability. Moreover, such synthesized graphene oxide nanosheets were further demonstrated to be biocompatible with human retinal pigment epithelium cells, making them promising candidates for in vivo clinical use. Poly(ethylene glycol) (PEG) hydrogel, a biomaterial, has also proved to be an excellent matrix for the encapsulation of enzymes. Additionally, PEG is known to repel proteins or cells, and is commonly employed to prevent surface fouling. Taking advantage of the dual functionality of a micropatterned PEG hydrogel structure, Yan et al. (43) juxtaposed cells next to enzymes entrapped in non-fouling matrices and detected the local production of hydrogen peroxide (H_2O_2) after mitogenic stimulation of macrophages in an optical manner. Using a similar concept, Matharu et al. (44) established an electrochemical sensor that monitors the oxidative stress generated by ethanol-injured hepatocytes.

5. DETECTING PROTEASE RELEASE FROM CELLS

Instead of employing enzymes as biorecognition elements, this variant of the biosensor detects enzymes as the targeted analyte. Of particular interest here is the cellular secretion of proteases, enzymes that remodel the extracellular matrix (ECM). Matrix metalloproteinases (MMPs) are proteases closely associated with the migration and invasion of cancer cells. Detection methods leverage the native capacity of proteases to cleave substrate peptides. For example, Mok et al. (45) developed a stimuli-responsive PEG delivery system and placed it in the vicinity of tumor tissues and nanoparticles released upon MMP-2-mediated cleavage of peptides in the gel. Also taking advantage of the protease–peptide interaction, Shin et al. (46) established an electrochemical sensor that detected MMP-9 by employing redox-labeled peptide on micropatterned electrodes.

A detection limit of 60 pM was achieved and the sensor was demonstrated to be capable of detecting signals from approximately 400 monocytes within 10 minutes. Later, Son et al. (47) developed a smart hydrogel that incorporated a cleavable peptide substrate coupled with a FRET pair (fluorescein isothiocyanate and DABCYL acid). Hydrogel microwells were used to define the cell adhesion area, and walls with sensing molecules were incorporated into the microwells to enable MMP-9 to be detected. MMP-9 released from as few as 11 cells has been detected (47).

6. SINGLE-CELL ANALYSIS

Traditional biosensors evaluate the average properties of a large population of cells and do not account for subsets of cells that function differently from the majority of the population. However, one could argue that these unusual cells, hidden within the predominant phenotype, are the most interesting and informative in terms of diagnosing and preventing disease. Flow cytometry and ELISpot are the gold standards of single-cell analysis (48). However, these commercial approaches do not completely satisfy all the needs of single-cell analysis and are particularly limiting in cases in which observations of specific single cells is beneficial. Below, we briefly review some aspects of single-cell analysis being addressed by the bioengineering community including the capture of cells, the retrieval of cells, and the analysis of secretions of single cells.

6.1. Single-Cell Capture

During the past decade, there has been a concerted effort by the bioengineering community to develop microtechnologies and biosensors for single-cell analysis; these technologies include microwells, microtubes, micropallets, microdroplets, and integrated microfluidic chips.

6.1.1. Microwells. Passively confining cells inside a microwell is one of the most prevalent methods used for single-cell assays due to its simplicity. To create a cell-friendly microenvironment, microwell arrays are typically composed of biocompatible materials such as PDMS (49–51), PEG (52, 53), collagen (54), agarose (55), or silicon (56). PDMS, collagen, and agarose-based microarrays can be fabricated via soft lithography approaches. Arrays of PEG hydrogel microwells may be created by using a photolithographic-like UV exposure with a photomask, or by using a soft lithography method.

Because cells are randomly trapped inside the microwells by gravity, the design of a microwell array focuses on improving the percentage of microwells containing a single cell (referred to as single-cell capture efficiency). Rettig & Folch (49) performed an optimization study of microwell arrays with high single-cell capture efficiency by varying the dimensions of the microwells (**Figure 3*a***). The optimal single-cell capture efficiency was obtained with a height-to-depth ratio of approximately 1; the capture efficiency was approximately 85% for adherent fibroblasts and approximately 92% for nonadherent rat basophilic leukemia-1 (RBL-1) cells. The randomness of single-cell capture in microwells may be reduced by modifying the bottom layer of the microwell with cell surface-binding antibodies, ligands, or peptides. When non-fouling hydrogel microwells were integrated with an underlying antibody-decorated hydrogel layer, single T cells were captured from a heterogeneous leukocyte suspension with a high single-cell capture efficiency of greater than 90% (52).

Microengraving, a technique that combines PDMS microwell arrays for single-cell capture and an immunoassay, was introduced by the Love group (57–59) and has been extensively used to detect various proteins secreted from single cells. In this technique, once single cells are trapped within the microwells, a glass slide modified with capture antibodies is placed on top of the

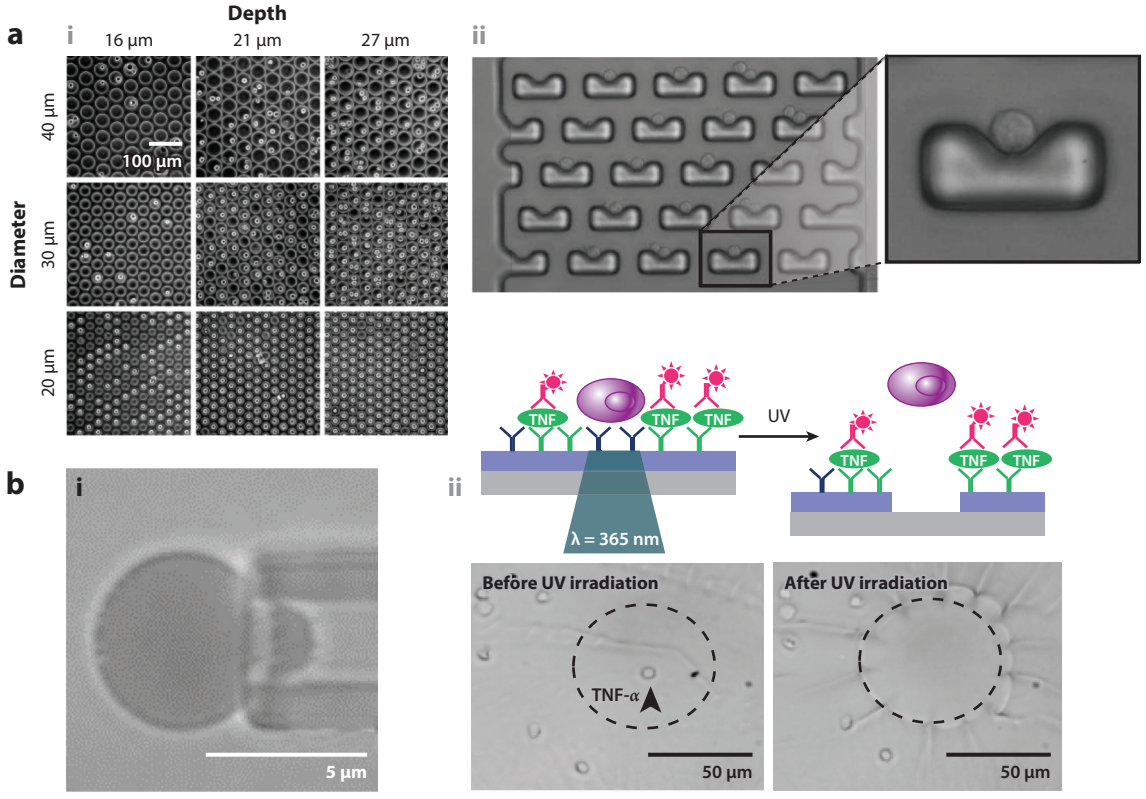

Figure 3

Single-cell handling for a single-cell assay. (*a*) Single-cell capture technologies. (*i*) Microwell arrays of different diameters (20–40 µm) and depths (16–27 µm) containing single rat basophilic leukemia-1 cells (49); (*ii*) a microtrap array (height, 40 µm; offset, 2 µm) with trapped single HeLa cells (average diameter, 15 µm) (71). (*b*) Selective single-cell retrieval methods. (*i*) A micropipette with an aspirated single, swollen red cell (76); (*ii*) retrieval of a single cell producing tumor necrosis factor-α (TNF-α) (*arrowhead*) from photosensitive hydrogels activated by UV irradiation (90).

microwell array, forming confined microchambers containing single cells. Cell-secreted proteins are captured on the antibody-modified glass slide, and then the slide is removed and stained with fluorescent-labeled secondary antibodies.

6.1.2. Microtubes and micropallets. Various microstructures, including microtubes and micropallets, have also been employed to confine single cells within 3D scaffolds. Microtubes, which can serve as label-free on-chip sensing devices, are prepared via a generic approach using positive photoresist as a sacrificial material (60, 61). A silicon monoxide–silicon dioxide ($SiO–SiO_2$) nanomembrane bilayer is deposited onto a circular-patterned photoresist layer using electron-beam deposition. Removing the photoresist with acetone leads the $SiO–SiO_2$ layer to roll up and form a microtube with a diameter of 6–10 µm. A micropipette used with a micromanipulator system was able to aspirate a single NIH 3T3 fibroblast cell into a microtube (12). Although this use was interesting, more will need to be done to make this approach amenable to high-throughput single-cell analysis.

Salazar et al. (62) reported using micropallets to selectively separate single cells. An array of micropallets composed of epoxy-based SU-8 photoresist was prepared on a glass slide via

photolithography, and single cells were adhered to the top surface of fibronectin- or collagen-coated micropallets (diameter, 30–40 μm). Micropallets with single cells were detached from a glass slide using focused laser-induced plasma (2 μJ), and collected for further cell culture (lasting up to 4 days). A similar microstructure, a microblock consisting of an enzymatically cleavable hydrogel, was utilized to track individual cells with stochastic particle barcoding (63). In this approach a solution of PEG diacrylate polymer, fluorescent coding beads, and single cells was poured into PDMS microwell arrays and sealed with a pH-sensitive sacrificial layer. After photopolymerization of the hydrogel, the sacrificial layer was dissolved, releasing the hydrogel microblocks containing single cells. The number, color, and position of the fluorescent beads could be used to identify and re-identify single cells in each microblock, with re-identification accuracies of 96% ± 2%.

6.1.3. Microdroplets. Monodisperse microdroplets can be continuously generated in a high-throughput manner (>1,000/s) in microfluidic devices using flow-focusing or break-up at T junctions. However, various factors make controlling the distribution of cells inside the droplets challenging; these factors include the concentration of the cell solution, the design and dimensions of the microfluidic device, the flow rate, and the need to determine the number of droplets that contain a single cell. The distribution of droplets encapsulating single cells (positive droplets) follow a Poisson distribution, often producing low capture efficiency for single cells (30–40%) (64). This efficiency may be increased to 80% by sorting cell-containing droplets using technology that is similar to flow cytometry (65). Another approach may involve arranging the cells in a microfluidic channel to match the frequency of droplet formation and cell entry into the nozzle of a microfluidic device (66).

Aqueous droplets containing single cells with reporter molecules, such as enzymes (67) or peptides (68, 69), have been used to sense cellular secretory activities. Because secreted proteins are confined inside droplets dispersed in an oil phase, crosstalk between neighboring droplets encapsulating single cells is completely inhibited, and the signal is intensified due to the small volume of individual droplets. Droplets can be made out of polymer or gel so that they do not need to remain in an oil phase. Single cells and recognition elements (e.g., antibodies or antigens) have been encapsulated in diffusion-controllable alginate droplets to monitor the secretion of antibodies from single cells (70).

6.1.4. Microstructured microfluidic chips. Microfluidics has become an important technology for enabling single-cell analysis. Microfluidic devices are frequently integrated with microstructures (e.g., microwells, microtubes, micropallets, and droplets) to provide consummate control over the cellular microenvironment with fast exchange of reagents. In this section, we emphasize microstructured microfluidic chips that have microtraps (71, 72) or pneumatically controllable microchambers (73, 74).

An interesting microfluidic-based single-cell culture chip has been described by DiCarlo et al. (**Figure 3*a*,*b***) (71). This device allowed them to capture one HeLa cell (from a human cervical carcinoma cell line) per trap with greater than 85% capture efficiency. After optimizing the trap's dimensions, and the location and spacing among traps, a capture efficiency of greater than 95% was achieved in trap arrays with a density of 860 traps/mm^2 (72).

The Quake group (75) introduced multilayer, soft lithography techniques to fabricate a pneumatically controllable microfluidic device. Also, a reconfigurable microfluidic device with a reversibly collapsible, micropatterned membrane has been employed to confine single cells inside microcompartments (73, 74). Once the cells have been captured inside the microchannel, a membrane containing a valve (73) or a microchamber (74) descended on the substrate to isolate

individual cells, thereby preventing crosstalk between adjacent single cells and, thus, amplifying the signals.

6.2. Cell Retrieval

It is often beneficial to capture a single cell, analyze its activity, and then retrieve a cell for downstream processing or analysis. Single-cell retrieval may rely on specialized instrumentation, for example, micropipettes and micromanipulators (**Figure 1*b***, subpanel *i*) (76, 77) or on laser-capture microdissection (78).

However, there is an interest in shifting from reliance on high-precision instruments toward developing smart substrates that may be activated locally and specifically. A number of dynamic substrates employing electrical potential (79–84), chemical composition (85–87), or light irradiation (88–90) have been introduced to retrieve cells. Although these are promising, electrochemical detachment strategies are somewhat challenging to adapt to retrieve specific, single cells. Our lab has previously demonstrated the release of a small group of cells from micropatterned, individually addressable electrodes (82, 83). Although shrinking such electrodes to accommodate single cells is relatively straightforward, statistically meaningful single-cell analysis and retrieval would necessitate the use of a high-density array of several thousand electrodes. This becomes somewhat more challenging. In our opinion, a more promising direction will be to use light-sensitive substrates to retrieve cells. This strategy would require a high-density array of micropatterned electrodes that could be addressed sequentially to change their surface properties and release cells. In this regard, the Allbritton lab (88, 89) developed micropallet arrays that can be used to culture cells. The adhesion of individual micropallets to the substrate can be disrupted by laser light, which can be used to dislodge a pallet with immobilized cells. Another light-directed cell-retrieval strategy has been demonstrated (80, 85, 90, 91),which employed photosensitive hydrogels impregnated with cell-adhesive peptide motifs or antibodies. As seen in **Figure 3*b***, subpanel *ii*, a specific spot of photoactive hydrogels can be degraded by UV light (365–405 nm) from a fluorescence microscope, thus releasing a single cell which had been adhered to the exposed hydrogel spot.

6.3. Analysis of Cell-Secreted Products

Products secreted by cells, including antibodies, cytokines, enzymes, and reactive oxygen species, are involved in cell differentiation, migration, proliferation, and response to injury or infection. Clearly, being able to detect these secreted molecules with single-cell resolution has important implications for basic biology and for disease diagnosis.

6.3.1. Antibody release from cells. Monoclonal antibodies are important for developing therapeutic and immunoassay applications (92, 93). Identifying clones that produce a desired antibody is a slow and relatively complicated process involving limited dilution of single cells in microtiter plates. Microfabrication techniques allow well geometry to shrink, increase the number of wells per unit area, and thus allow signals to be detected sooner than in macroscale multiwell plates. Microwell arrays (56, 57) and microdroplets (68, 70, 94) have been employed in early screening of antibody secretion to reduce the time and labor related to the expansion of individual clones.

A microengraving method was used by Love et al. (57) to detect antibody secretions from single cells. Secreted antibodies (anti-chicken ovalbumin and anti-mouse H-2K^b) were successfully detected from hybridoma cells cultured in PDMS microwells (**Figure 4*a***). Jin et al. (56) used arrays of functionalized wells to detect single cells producing antibodies. This study detected single cells secreting antibodies specific to the hepatitis B virus surface antigen (>15% of cells). Compared

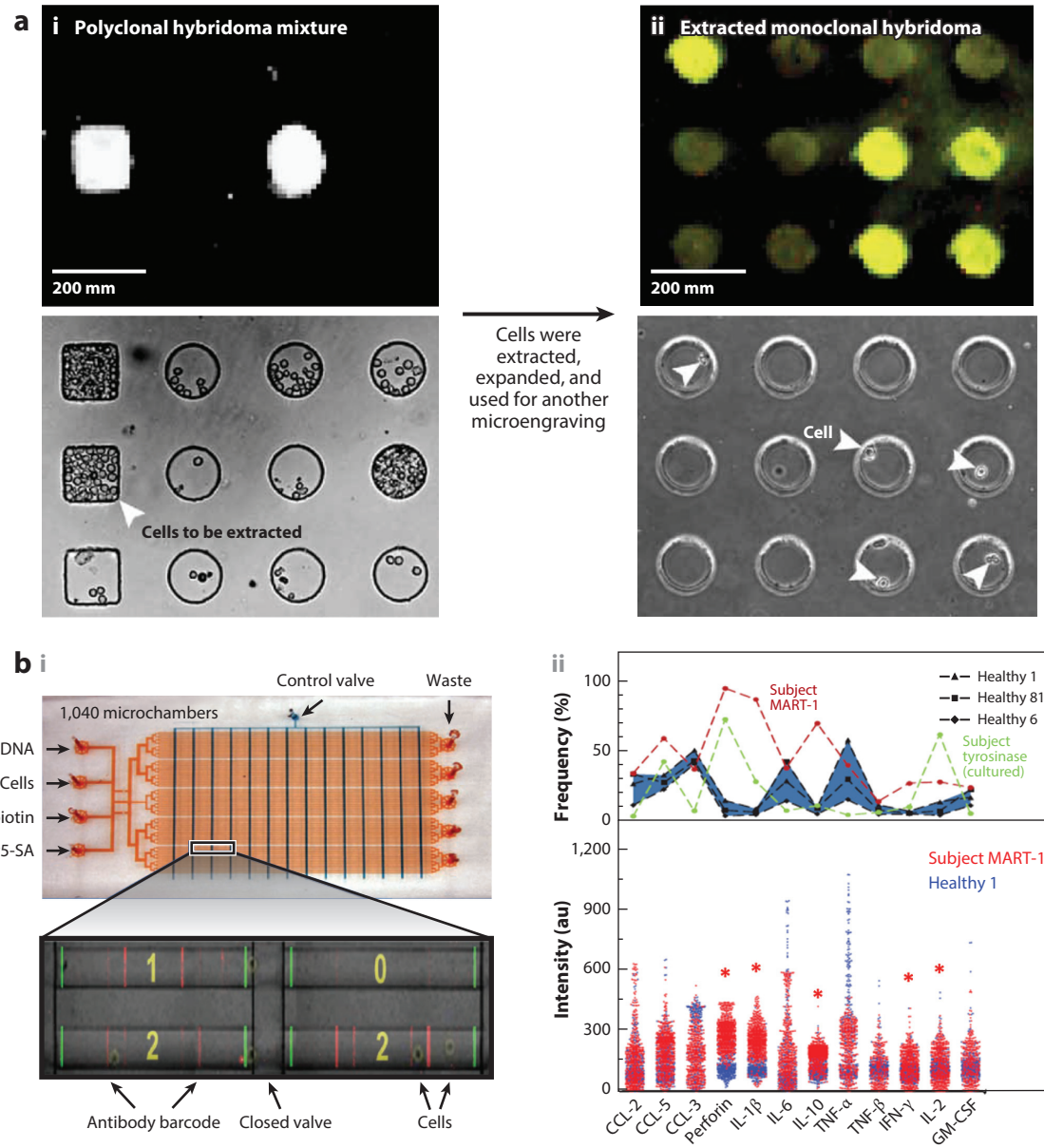

Figure 4

(*a*) Representative single-cell assays. (*i*) Selection of antibody-secreting cells (ASCs) from a polyclonal hybridoma; (*ii*) confirmation of the specificity of anti-mouse H-2K^{b} antibodies secreted from extracted, expanded ASCs using microwell arrays (fluorescence microscopy images of secreted antibodies and phase-contrast microscopic images of the corresponding microwells containing cells) (57). (*b*) Single-cell cytokine secretome analysis using a barcode chip (73). (*i*) Photograph showing a single-cell barcode chip designed with flow channels (*red*) and control channels (*blue*), and a microscopic image showing cells confined in the microchambers and the developed assay barcodes. Numbers indicate the number of cells per microchamber. (*ii*) Comparison of melanoma-associated antigen-specific T cell receptor transgenic T cells and healthy donor CD8^{+} T cells. Abbreviations: Ab, antibody; GM-CSF, granulocyte–macrophage colony-stimulating factor; IL, interleukin; TNF, tumor necrosis factor.

with the conventional ELISpot assay, this strategy allowed four times more antibody-producing cells to be detected.

Instead of using microwells fabricated on 2D surfaces, researchers are using microdroplets as compartments to entrap single cells and detect secreted factors (68). Using this technique, up to 300,000 single hybridoma cells were sorted according to their antibody inhibitory function on angiotensin-converting enzyme-1 (ACE-1) (sorting rate approximately 5×10^4 cells/h). Single hybridoma cells and recombinant ACE-1 were encapsulated into droplets and fused with ACE-1-specific FRET peptides, which generate a fluorescence signal upon hydrolysis by ACE-1. Once the antibody-secreting cells secreted ACE-1 inhibitory antibody 4E3, the enzymatic activity of ACE-1 was inhibited, resulting in the low fluorescence of peptides. Akbari & Pirbodaghi (70) reported a microparticle-based immunoassay that could be used to screen anti-TNF-α antibody-secreting cells from a mixture of hybridoma cells. Single cells were encapsulated, along with a secondary antibody complex and fluorescent antigen, inside nanoporous alginate microparticles, which controlled diffusion based on molecular weight. The diffusion of the secreted antibodies was prevented by binding to antigens and secondary antibody complexes, while the unbound fluorescent antigens diffused out, reducing the background signal and enhancing the efficiency of detection.

6.3.2. Detecting cytokine secretion. Cytokines are small proteins used to signal important events associated with immune response and inflammation. Cytokine production is heterogeneous. For example, only a small subset of T cells responds to antigenic stimulation by producing cytokines such as IFN-γ and IL-2 (95, 96). The Heath group (73) introduced a single-cell barcode chip to assess functional heterogeneity in human T cells. Cells were loaded into microchambers (approximately 3 nL) that were divided by vertical valves on the top PDMS layer; secreted cytokines were detected using an immunosandwich assay from a spatially encoded antibody barcode. Twelve different kinds of proteins were assayed from healthy donor CD8+ T cells and from T cells engineered to recognize melanoma-specific antigen (MART-1). This device was used to assess the functionality of T cells used for immunotherapy applications.

A simpler cytokine assay kit, which did not require sophisticated fluidics, has been developed by Lu et al. (97). The secretion profiles were demonstrated for 14 different proteins, including cytokines, chemokines, and growth factors, from single U87 cells (glioblastoma multiforme), U937 monocytes (leukemic lymphoma), and A549 cells (lung carcinoma). Notably, the authors simultaneously measured protein secretion and cellular migratory properties, reporting that highly migratory cells are statistically correlated with high expression of IL-8 ($P < 0.01$).

Recently, a microfabricated platform was developed for real-time detection of cytokine secretion using total internal reflection fluorescence microscopy (50). IL-1β secreted from stimulated human peripheral blood monocytes was monitored at 1-minute intervals, demonstrating cell-to-cell heterogeneity in the onset of cytokine secretion.

6.3.3. Monitoring enzymatic activity in single cells. Enzymes are important indicators of cell signaling and may also be involved during cell migration and the metastasis of cancer cells. Eyer et al. (74) used reconfigurable microfluidic devices to trap single cells, lyse them, and analyze concentrations of GAPDH, the enzyme that is commonly used as a housekeeping gene in PCR analysis. Similar concentrations of this enzyme were detected in two different cell types, with limited cell-to-cell variability. This confirmed that GAPDH is indeed a good choice for a housekeeping marker.

It is often more informative to analyze the activity of an enzyme rather than its concentration. Juul et al. (98) employed rolling-circle amplification to quantify enzyme activity in single HEK293

cells encapsulated in microdroplets. In this technique, the enzymes Flp recombinase and hTopl convert linear DNA sensors (substrates) to circular products that are templates for rolling-circle amplification, generating approximately 10^3 repeat products that are hybridized with fluorescent probes and visualized in a microscope. The Han group (99) developed a microfluidic device to selectively lyse specific, single cells from an adherent monolayer culture in order to analyze Akt kinase activity. By employing a peptide substrate, Akt activity was monitored with high sensitivity.

7. NANOSTRUCTURED BIOSENSORS FOR CELL ANALYSIS

Using nanostructures, such as nanowires, to analyze cells offers interesting advantages in terms of geometry, electrical conductivity, and high surface-area-to-volume ratio. Such biosensors may be inserted into live cells to detect intracellular processes. The high aspect ratios and small geometries of nanostructured biosensors also have benefits for monitoring extracellular events. Of the various types of nanostructures, silicon nanowires (SiNWs) have seen the widest use in the biomedical field (100, 101).

7.1. Fabricating Nanostructured Sensors and Interfacing Them with Cells

Devices have been constructed around single, or several dispersed, SiNWs (102, 103), and methods have been developed to manipulate as-grown nanowires into geometries amenable to fabricating a large number of devices simultaneously (104, 105). SiNWs use a vapor-liquid-solid growth mechanism, which provides wires in diameters ranging from micrometers to millimeters (106, 107). Vapor-liquid-solid growth using chemical vapor deposition can produce epitaxially aligned, single-crystalline wires (108). Gold nanoparticles can act as nucleation sites for crystallization, and produce 1D growth of SiNWs (109). It is worth noting that similar strategies have been used to fabricate nanowires from materials other than silicon. For example, group II–VI semiconductors may be synthesized using the chemical vapor deposition process with appropriate nanocluster catalysts and growth temperatures and pressures (110, 111). Top-down approaches, such as wet chemical etching with hydrofluoric acid and silver nitrate, can also be used to create a surface of silicon nanopillars, with diameters ranging to hundreds of nanometers (112).

The features of cell surfaces are of similar scale to nanostructure-fabricated surfaces, which may offer advantages for capturing cells (113). A nanowire can effectively increase surface contact with extracellular features, thereby improving capture efficiency (114). Capture probes, including antibodies (112, 115), aptamers (116), and streptavidin (100, 117), have been immobilized on SiNW surfaces. Such surfaces have been used to capture circulating tumor cells (112, 115) and CD4+ T lymphocytes (116–118). The density and diameter of nanowires have been shown to be important parameters for cell adhesion and spreading on nanostructured surfaces (119).

Once the cells are adherent, they may be pierced by nanowires in a way that is nondestructive and allows processes inside the living cells to be monitored. In this regard, Shalek et al. (120) demonstrated that vertical SiNWs can be used to spatially localize cells, penetrate cell membranes, and release molecules from nanowires into the cytoplasm. Using this technique, various biomolecules, including small interfering RNA, peptides, DNA, and proteins, can be delivered into primary neurons and fibroblasts (120). In a follow-up study, the same group used vertical SiNWs for delivery and functional interrogation of diverse immune cells. By optimizing the dimensions of the nanowire, they demonstrated that nanowires could consistently penetrate cellular membranes without activating an immune response or interfering with normal immune sensing. This approach has been used to investigate the role of the Wnt signaling pathway in chronic lymphocytic leukemia (121). In another development, the Melosh group (122) described using

a nanostraw platform to mimic transmembrane protein channels. The nanostraws were fabricated using a porous polycarbonate membrane—which is widely used for cell culture and water purification–as a template. An alumina coating was deposited on the surface of the membrane and within the nanopore interior; this was followed by reactive ion etching, which was used to control the thickness and height of the nanostraw. The nanostraw membrane was supported over a fluidic chamber, and the delivery solution was diffused through the nanostraw into a cell, thereby providing a permanent fluidic pipeline into the cell for direct cytosolic access. Small molecules and ions were successfully transported into the cytosol with, respectively, 40% and 70% efficiency; green fluorescent protein plasmids were successfully delivered and expressed (122).

7.2. Extracellular Biosensors Based on Silicon Nanowires

Nanostructured surfaces are particularly attractive in electrochemistry where such surfaces enhance diffusion, decrease response times, and improve electrode sensitivity (123). The Kelley group (124) used the electrodeposition of noble metals to develop 3D nanowire electrodes; the noble metals were deposited onto a template created by a passivation layer introduced onto the surface of a silicon or glass chip patterned with gold leads. The probe nucleic acids were immobilized on the nanowire surface and used in conjunction with an electrocatalytic reporter system that relies on the interaction of soluble $Ru(NH_3)_6^{3+}$ with negatively charged nucleic acids that accumulate at the sensor surface, and on the regeneration of electrochemically reduced Ru(II) by $Fe(CN)_6^{3-}$. Gold nanowires were electrodeposited on micrometer-sized apertures from the bottom of the gold electrode, and a fine nanostructure was thus generated. By varying the deposition time, the size and complexity of the nanostructure could be effectively controlled. An attomolar limit was achieved for the detection of bacterial RNA (125). By varying the size of the sensor, the composition of the material, and the probe density, the analytical sensitivity was fine-tuned and the detection limits were adjusted by a factor of 100. A more highly structured coating yields the most sensitive detectors (126). A nanostructured microelectrode has also been integrated into a microchip-based platform and coupled with electrochemistry to detect RNA from the lysate of as few as 10 cancer cells (127). The device is cost effective, combines ultrasensitive detection with straightforward sample processing, and has a sample-to-answer time of 30 minutes. The clinically relevant detection limit of 1 colony-forming unit per microliter of intercellular RNA was achieved, indicating its potential success as a point-of-care diagnostic device (128).

7.3. Intracellular Biosensors Enabled by Silicon Nanowires

The cell membrane is decorated with a large number of proteins and receptors that are responsible for transporting molecules, ions, and signals from outside into the cell. Functionalizing nanowires with cell lipid bilayers provides an opportunity for interfering with biological signaling mechanisms at the nanostructure level (129). The Noy group (130) assembled a continuous lipid bilayer shell around a hydrophilic polysilicon nanowire, developing a mobile structure with a low concentration of defects. In a follow-up study, they placed alamethicin, a voltage-gated ion channel, in the lipid shell, and used an electric field from a nanowire FET to open and close the protein pores in the bilayer. The alamethicin pores opened at positive voltages, and the nanowire FETs could respond to changes in the pH of the solution flowing through the cell (131). The nanowire FETs were also integrated into a membrane protein ion pump (Na^+/K^+-ATPase) to monitor ion transfer across the cell membrane (132).

The cell membrane is a self-healing barrier and virtually impenetrable to ions and small molecules. It is interesting to gain a window into a cell in order to monitor processes that cannot

be detected from the outside. Historically, biology has relied on gene-reporter technologies (133). The field of biosensors may offer alternatives to the real-time nonfluorescence detection of intracellular processes. The ability to deliver a nanowire electrode into a single cell to make electrical measurements inside single cells or throughout the entire 3D space of a tissue has many important implications for electrophysiology and biomedical sciences (134). Nanowire FETs can function in the sub-10-nm range: their exceptionally small size enables them to function as mechanically noninvasive probes that enter cells through endocytic pathways and then become lodged in the cytoplasm (135, 136). The dynamic flow of ions inside cells can generate a spatially defined field potential that can function as a gate signal to modulate the electrical output in nanowire FET devices. Tian et al. (137, 138) developed a kinked nanowire FET device to record intracellular signals, representing the first time that a nanotransistor was positioned inside a cell (**Figure 5*a***). The electrical recordings of spontaneously beating cardiomyocytes demonstrated that the 3D nanowire FET probes continuously monitored extra- to intracellular signals during cellular uptake (**Figure 5*b***). The Lieber group (139) developed a nanotube FET, in which a branched SiO_2 nanotube was integrated on top of a nanowire FET. This nanotube penetrated the cell membrane to bring the cytosol into contact with the extracellular FET to enable intracellular recording of transmembrane potential from a single cardiomyocyte (**Figure 5*c***). An intracellular probe, known as an active nanotube transistor (ANTT), was fabricated by attaching the source and drain contacts of a FET to one end of a silicon or semiconductor nanotube and electrically isolating these from the surrounding medium. Multiplexed arrays of ANTT devices were fabricated by assembling multiple ANTTs at the ends of single probes, which enabled simultaneous recording of full amplitude intracellular action potentials from single cells (**Figure 5*d***) (140).

In addition to detecting intercellular potentials, the SiNW array has also been used to measure intercellular enzymatic reactions. Na et al. (141) developed a sandwich nanowire array to probe various enzymatic reactions in living cells, including the activities of proteases, phosphatases, and protein kinases; they also studied the intracellular signal transduction pathway (141). Live HeLa cells were sandwiched between two vertical SiNW arrays. The first nanowire array was used to locally immobilize cells; the second nanowire array was used to immobilize enzymatic substrates tagged with a fluorescent dye where they reacted with the intracellular enzyme when the second nanowire array penetrated the cellular membranes without affecting the cells' viability and function.

7.4. Three-Dimensional Nanoelectronic Scaffolds

The fabrication of nanowire arrays has led to new research that merges nanoelectronics with biological systems in three dimensions. For example, nanowire FETs have been implanted into flexible, stretchable matrices that conform to tissue surfaces (123, 142–144). In one example, a single nanowire FET was metallized, passivated with epoxy, and knotted together using lithography patterning to form 3D nanowire FET matrices (that is, nanoelectronic scaffolds). Synthetic or natural macroporous ECMs were then integrated into the nanoelectronic scaffolds through biological processes. The combination of nanoelectronic scaffolds and ECMs provided ECMs with electrical sensory functions and nanoelectronic scaffolds with biochemical environments that were suitable for tissue culture (**Figure 6*a***) (145). Two nanoelectronics scaffolds (one reticular and one mesh) were designed and contained similar components. Briefly, a layer of SU-8 negative resist was coated on a nickel sacrificial layer, and a solution with a nanowire FET was deposited onto the SU-8 layer and allowed to evaporate; then the SU-8 layer was patterned using lithography to immobilize the nanowires and provide the basic framework for the nanoelectronic scaffolds. Another SU-8 layer was deposited and lithographically defined as the final, upper passivation

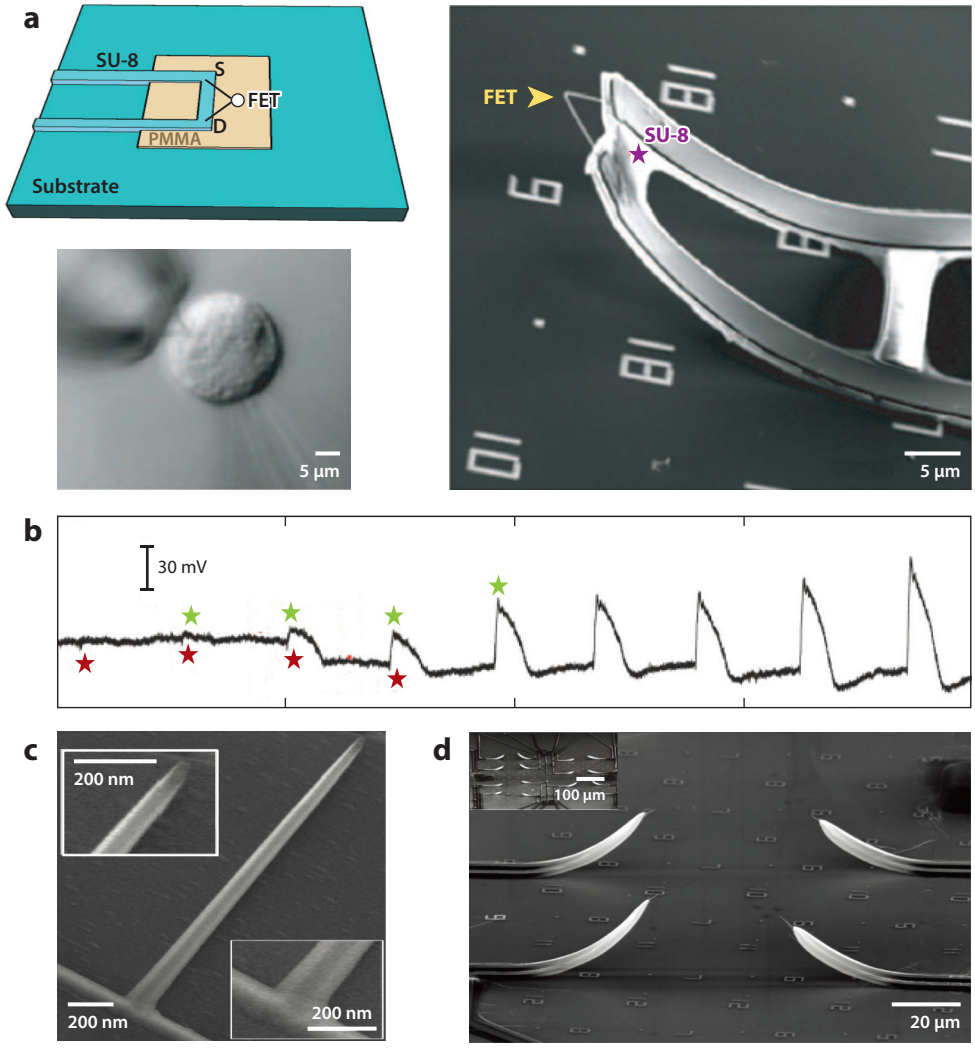

Figure 5

(*a*, *left*, *top*) Fabrication of a 3D kinked nanowire field-effect transistor (FET) probe. Poly(methyl methacrylate) (PMMA) and an SU-8 micropattern serve as the flexible support for the device. (*right*) Image from scanning electron microscopy (SEM): the nanowire FET (*yellow arrowhead*) and SU-8 (*purple star*). (*left*, *bottom*) Differential interference contrast microscopy images show the kinked nanowire probe contracted and with an internalized HL-1 cell (137). (*b*) Electrical recording from beating cardiomyocytes with the transition from extracellular to intracellular recording during cellular entrance of a nanowire FET probe. Initial extracellular signals gradually disappear (*red stars*), then the intracellular signal appears and reaches a steady state (*green stars*) (137). (*c*) SEM image of nanotube FETs with a silicon nanotube connected to the silicon nanowire FET. The insets are magnified images of the top and bottom of the nanotube (139). (*d*) SEM image of an active nanotube transistor probe array (140). Panels modified from the aforementioned references with permission.

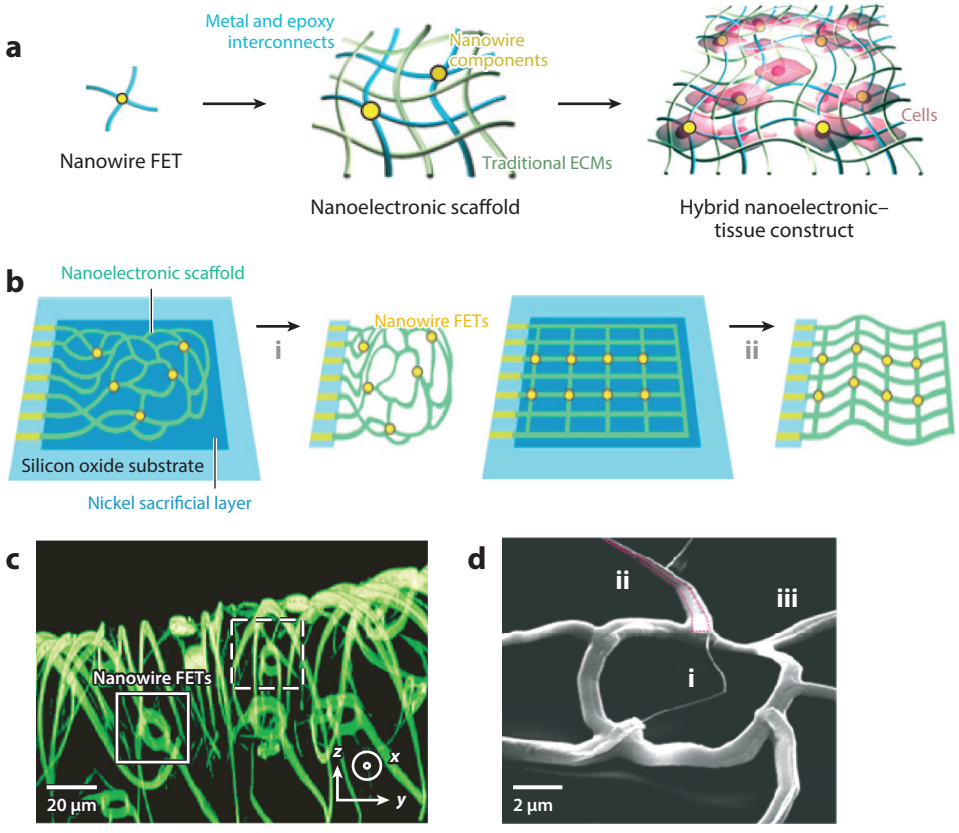

Figure 6

(*a*) Schematic representation of the integration of nanoelectronics with cells and tissues. A 3D nanoelectronic scaffold was formed by self-organization or manual organization of a nanowire field-effect transistor (FET), and this was hybridized with traditional extracellular matrices (ECMs): nanowire components (*yellow dots*), metal and epoxy interconnects (*blue ribbons*), traditional ECMs (*green ribbons*), and cells (*pink shading*). (*b*) Device fabrication schematics. (*i*) Reticular nanowire FET devices and (*ii*) mesh nanowire FET devices. Silicon oxide substrates are shown in light blue, nickel sacrificial layers are shown in blue, nanoelectronic scaffolds are shown in green, and individual nanowire FETs appear as yellow dots. (*c*) A 3D reconstructed confocal fluorescence micrograph of a reticular nanoelectronic scaffold. The scaffold was labeled with rhodamine 6G: Two nanowire FETs located on different planes are represented by the solid and dashed squares. (*d*) Image from scanning electron microscopy of a nanowire FET within a reticular scaffold, showing (*i*) the kinked nanowire, (*ii*, *dashed lines*) the metallic interconnects, and (*iii*) the SU-8 backbone. Figure modified from Reference 145 with permission.

layer on the scaffold. Reticular scaffolds (**Figure 6*b***, subpanel *i*) were made using electron beam lithography, and self-organization created a random network of 3D features that mimicked the size, scale, and morphology of submicron-sized features of the ECM. Mesh scaffolds (**Figure 6*b***, subpanel *ii*) were made to have a regular structure using photolithography, and to be similar to the ECM of the ventricular myocardium. The free-standing nanoelectronic scaffolds were later rolled or folded to form 3D networks that were highly flexible, biocompatible, and had high porosity (>99%). Reconstructed 3D confocal fluorescence images of the reticular scaffolds showed that the 3D framework had a highly curvilinear and interconnected structure (**Figure 6*c***). The nanowire FETs within the scaffold spanned separations of 7.3–324 μm in three dimensions, and the heights

of the scaffolds were less than 300 μm. Scanning electron microscopy of the nanoelectronic scaffolds revealed kinked nanowire FETs (about 80 nm in diameter), and metallic interconnects (about 0.7 μm wide) contained within the SU-8 backbone (about 1 μm wide) (**Figure 2*d***).

Finally, cells were cultured within the nanoelectronic scaffolds to yield 3D hybrid nanoelectronic–tissue constructs. Neurons, cardiomyocytes, and smooth muscle cells were merged with the nanoelectronics in three dimensions, and local electrical activity was monitored in real time. The potential of using nanoelectronic scaffolds to test responses of cardiac cultures to drug candidates was demonstrated (145). Measurements from the same nanowire FET device showed a two-fold increase in contraction frequency after a cardiac contraction stimulated by application of a drug compared with before the application of a drug. Multiplexing measurements made with a reticular nanoelectronic scaffold–neural construct also showed that the 3D response of drug stimulation could be monitored (145), suggesting the potential for using 3D nanoelectronic scaffolds with a cell-culture platform for in vitro pharmacological studies. Additionally, simultaneous recordings from four nanowire FETs separated by up to 6.8 mm in a nanoelectronic scaffold–cardiac construct demonstrated multiplexed sensing of a coherently beating cardiac patch with sub-millisecond time resolution, which could allow the nanoelectronic scaffolds to map cardiac and other electrical activities of synthetic tissues over entire constructs at high densities in three dimensions.

8. CONCLUSIONS AND PERSPECTIVES

Unfortunately, our article cannot incorporate all of the new and interesting developments in the field of biosensors. Instead, we have looked at the field of biosensors through the prism of our interest in cell analysis, but, even then, there is a large body of literature that is not captured in this review. First, we wanted to highlight some of the most important biorecognition elements being used in biosensors for cell analysis. Second, we have also described technologies for integrating cells with sensors, which is particularly important for analyzing single cells. Our third area of focus was on nanostructured biosensors. In terms of applications, there are clear benefits to using cell analysis for diagnosis. This is particularly true in immunology where cellular responses to antigens are frequently used diagnose infectious diseases, such as TB or HIV. We believe that as biosensors become more frequently used for cell analysis and find their way into the hands of clinicians and biologists, the diagnostic utility of functional cell analysis will also be demonstrated for cancer research, neurodegenerative disorders, and other pathologies.

Another use of biosensors that is particularly exciting to us is as research tools for improving understanding of cellular interactions and paracrine signaling. The scientific community and funding agencies are increasingly emphasizing the need to develop in vitro surrogates for important tissues and organs. This push is motivated by the complexity and cost of using animal models. Also, certain aspects of pathogenesis are too fleeting to be recorded in animal models and, therefore, in vitro analysis is warranted. Our vision is that biosensors will be integrated with cell-culture systems and will be used to dissect the signaling underlying tissue injury or regeneration. In general, we foresee that the use of biosensors for cell analysis will expand exponentially during the next several years and provide novel functionalities in the fields of biology and medicine.

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Errata

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