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Micro- and Nanotechnologies for Study of Cell Secretion

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diameter) fuse with the plasma membrane and release their content into the extracellular space. Constitutive exocytosis occurs constantly as a housekeeping activity to release proteins, metabolites, etc. Regulated exocytosis, on the other hand, is only triggered by specific stimuli to release signaling molecules. A classic example is Ca^{2+} triggered neurotransmitter secretion at neuronal synapses.

Assays of cell secretion are not new at all to biologists and have been indispensable to fundamental studies and diagnosis for decades,¹ but tremendous efforts have been continuously taken to develop new techniques in order to deal with the long-existing challenges for study of cell secretion, particularly, regulated or triggered exocytosis. Regulated exocytosis is temporally and spatially regulated by the orchestrated actions of a large variety of proteins and lipids.^{2,3} It is a highly dynamic process in which the secretory vesicles undertake the cascaded biochemical steps to reach and dock on cell membrane, mature to releasable state, and finally fuse with cell membrane to discharge the cargos upon triggering.^{4,5} Exocytosis of readily releasable vesicles in response to a stimulus can occur rapidly in a kinetic time constant of tens of milliseconds with a minimal delay of milliseconds.^{6,7} The quantal fusion between vesicular membrane and cell membrane takes place in just a few milliseconds,⁸ and secretory vesicles dock and fuse at defined membrane micro- or nanodomains (exocytic sites) where the exocytotic proteins such as soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) are clustered.⁹

Cell secretion is conventionally assayed using ensemble biochemical measurements from a large cell population with low temporal resolution. They are not able to resolve the fast kinetics of cell secretion, to reveal dynamic and heterogeneous behavior of individual cells, to reveal heterogeneous functions of cell membrane domains, or to detect a trace amount of released molecules (zepto-moles from single vesicle release). The emerging and rapidly evolving micro- and nanotechnologies are providing new tools to spatiotemporally resolve this critical and ubiquitous cell function, which are instrumental to fundamental studies, diagnosis, and drug discovery for secretion related diseases (e.g., neurological and metabolic diseases). In this article, we attempt to review the recent important advances for investigating cell secretion with improvement of sensitivity, temporal resolution, spatial resolution, or assay throughput. These technologies are developed by utilizing micro/nanosensing

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elements, micro/nanofabrication, novel detection principles, novel nanomaterials, or microfluidic process. Although we intend to include all the significant works in the field, some articles may still have been inadvertently left out. We apologize for these oversights and would like to stress that this article emphasize more, although not exclusively, on the important technical advances made from year 2005 to present and on the studies of the dynamic regulated (triggered) exocytosis in live cells which is more demanding on detection sensitivity and spatiotemporal resolution as compared to the demands for analysis of the slow constitutive (constant) exocytosis or static detection of bulk concentration.

■ CONVENTIONAL BIOCHEMICAL ASSAYS AND THEIR COUPLING WITH MICRO- AND NANOTECHNOLOGIES

In a biologist's lab, cell secretion is usually analyzed using biochemical methods including Western blot, enzyme-linked immunosorbent assay (ELISA), and radioimmunoassay.^{1,10,11} Although these methods are generally reliable, false detection may occur due to nonspecific binding to the substrates or nonspecific immunoreactions. Western blot analysis is considered as the most reliable since the molecular weight of the analyte can be determined to avoid false positives. On the other hand, ELISA and radioimmunoassay are highly sensitive and can provide quantitative analyses. All these methods require multi-step procedures (e.g., precollection of cell culture medium, sample concentration, or other treatments) and skillful experimenters (particularly for radioimmunoassay which involves radiation hazard). Mass spectroscopy¹² is another important, yet expensive, tool, particularly useful to obtain the profile of all secreted molecules and to identify unknown molecules from a less understood cell model. Although the aforementioned methods are and still will be indispensable for the study of cell secretion, they are of low-throughput and low temporal resolution (typically taking hours), are not possible for detection at single cell or subcellular level, and consume a large amount of samples and reagents.

Some of the limitations associated with the conventional biochemical assays can be alleviated in part by applying micro- and nanotechnologies. For example, microfluidic Western blot (μ WB) has recently been demonstrated.¹³ The μ WB chip assembled by incorporating a polydimethylsiloxane (PDMS) microfluidic network with the blotted polyvinylidene fluoride membrane can be used to simultaneously analyze multiple proteins in a single sample, and it requires only $\sim 1\%$ of the amount of antibody needed in conventional Western blot. In the work by Koster et al., microfluidic devices were used to encapsulate, incubate, and manipulate individual cells in picoliter droplets, where molecules secreted by the encapsulated cells can rapidly attain a detectable concentration for subsequent ELISA analysis.¹⁴ Miniaturized lab-on-a-chip for ELISA (ELISA-LOC) has been developed for detection of staphylococcal enterotoxin B (an exotoxin excreted by the *Staphylococcus aureus* bacterium).¹⁵ This ELISA-LOC device utilizes the large surface area of a carbon nanotube (CNT) matrix to improve antibody immobilization and, thus, the sensitivity of ELISA assays, and for handy analysis, it incorporates multiple functional components including: reagent loading fluidics, assay and detection wells, reagent removal fluidics, and a simple "surface tension" valve used to control the flow. Operated with a syringe without the requirement of any external power supply, the entire assay can be carried out in 2 h

with minimal sample handling or manipulation. Microchip based ELISA has also been demonstrated for trace detection of α -fetoprotein (a hepatocellular carcinoma biomarker) in human serum with a sensitivity of 1 pg/mL.¹⁶ As another example, mass spectroscopy can be performed on a single live cell using a nanospray ionization tip.^{17,18}

■ ELECTROCHEMICAL DETECTION USING MICRO- AND NANOELECTRODES

Many secretory compounds (e.g., epinephrine, norepinephrine, dopamine, serotonin, neuropeptide Y) can be readily oxidized on a voltage biased electrode. Therefore, secretion of these compounds can be electrochemically detected. In the amperometry mode, oxidative current is recorded at a fixed holding voltage. Integration of the amperometric current indicates the total amount of the released molecules. While in the voltammetry mode, the resulting current versus the rapidly varied voltages (voltammogram) can be used to identify the oxidized molecule based on its characteristic shape.

Microelectrodes. Pioneered by Wightman,¹⁹ carbon fiber microelectrodes (CFEs) with an uninsulated (active) flat tip of $\sim 5-10\ \mu\text{m}$ in diameter have now widely been used to electrochemically detect secretion from a single cell. With a CFE positioned on the cell surface, single vesicle release can be resolved as an amperometric current spike which reveals the amount of released molecules and real-time kinetics of the quantal exocytotic event. The readers may refer to a number of excellent reviews on this topic.²⁰⁻²³ CFEs have also been proved useful to detect neurotransmitters and monitor their concentration dynamics within neural tissue.²⁴ Although microelectrodes could be fabricated with metal materials such as platinum, carbon fiber is overwhelmingly favored due to its excellent electrochemical properties and, importantly, its mechanical properties (e.g., its stiffness ensures mechanical stability while being held onto a cell).

Nanoelectrodes. Continuous efforts have been taken to further reduce the size of the electrochemical electrodes in order to record at fine cellular structures (e.g., synapse—nanometer gap where two slender neurites meet), to provide higher spatial resolution for pinpointing exocytotic sites, to minimize the broadening of amperometric signal due to molecule diffusion, to decrease the noise level, and to avoid spike superimposition. Wightman's group used CFEs with a diameter of $\sim 1\ \mu\text{m}$ to detect secretion from a membrane area of $<1\ \mu\text{m}^2$ and demonstrated in chromaffin cells that vesicular exocytosis of catecholamines preferably occurs at active (hot) zones on the cell membrane colocalized with the sites of Ca^{2+} entry.^{25,26} Using similarly sized electrodes, it was also found by Paras et al. that only certain membrane regions of a pancreatic β -cell actively undergo exocytosis.²⁷

Wu and colleagues demonstrated the first carbon-fiber nanoelectrodes (tip diameter, $\sim 100\ \text{nm}$) which exhibit exceptionally low noise (thus higher sensitivity) and used them to amperometrically monitor exocytosis from PC12 cells with single-vesicle spatial resolution (Figure 1).²⁸ In their experiments, there was no detection in $\sim 70\%$ of the recordings, and only one to a few spikes were observed when the nanoelectrode was right above the fusion site, indicating that the majority of the cell surface is not active for exocytosis. They discovered that multiple fusion events could occur at the same site, and sequential release at those hot spots played the major role in the dopamine release from PC12

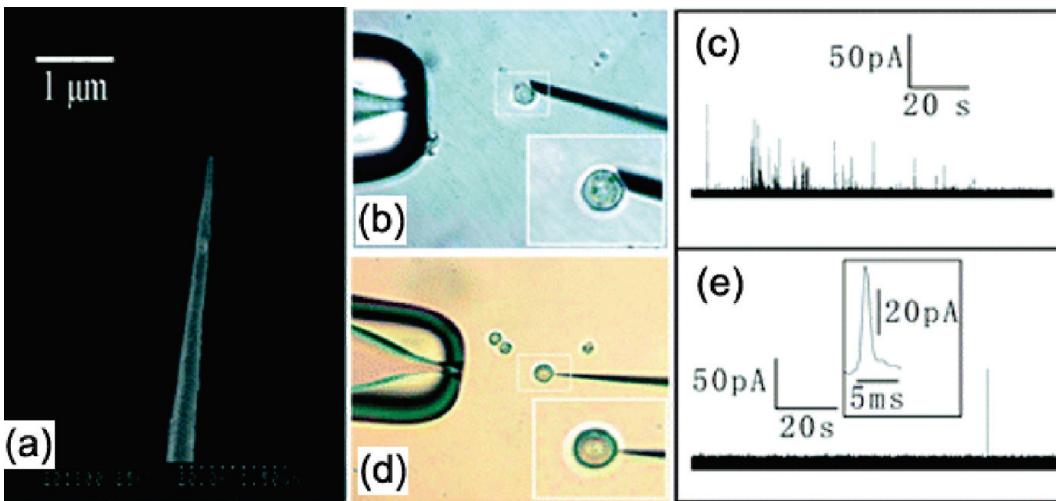


Figure 1. Amperometric monitoring of release from PC12 single cells by a microelectrode ($d = 5 \mu\text{m}$) and a nanoelectrode (tip diameter = ca. 100 nm). (a) SEM picture of a nanoelectrode; (b and d) photographs of actual microelectrode–cell and nanoelectrode–cell arrangements, respectively; (c) results from a carbon fiber microelectrode; (e) results from a nanoelectrode (ca. 100 nm). The current spikes are magnified in the inset. Adapted with permission from ref 28. Copyright 2005 American Chemical Society.

cells. It has also been shown by another group that amperometric spikes recorded by nanoelectrode ($\sim 500 \text{ nm}$) reflect the quantal fusion kinetic with better fidelity because the amperometric signals from commonly used large electrodes could be significantly broadened due to molecule diffusion from the fusion site to the electrode surface.²⁹ They showed that the half-width of the amperometric spikes recorded by the nanoelectrodes were smaller than that from the conventional microelectrodes, presumably due to reduced broadening effect. As demonstrated by Cheng et al., the sensitivity and spike-separation efficiency were also markedly improved using nanoelectrodes.³⁰

Detect Secretion of Nonoxidizable Substances. As compared with the biochemical assays, electrochemical detection based on micro- or nanoelectrodes provides submillisecond time resolution, single-vesicle release sensitivity, and feasibility to record at single cell or subcellular level, but an obvious limitation of this technique is that it is only applicable to oxidizable substances. An approach to tackle this issue is to load the secretory vesicles with exogenous oxidizable amines via endocytosis.³¹ It has been demonstrated that vesicles in a number of cell types (including hippocampal neurons, pituitary endocrine cells, pancreatic epithelial, AtT-20, and yeast cells) can be loaded with dopamine or serotonin for single-cell CFE amperometry.³² Detection of nonoxidizable substances can also be mediated by specific enzymes. For example, triggered L-glutamate secretion from hippocampal neurons can be detected by a bienzyme (horseradish peroxidase/L-glutamate oxidase) redox hydrogel modified microelectrode,³³ and histamine released from antigen dinitrophenylated bovine serum albumin (DNP-BSA) stimulated RBL-2H3 cells can be detected with an electrode modified with Os-polyvinylpyridine based mediator containing horseradish peroxidase (Os-gel-HRP) and histamine oxidase (HAOx).³⁴

Nanomaterial Derived Electrodes. Nanomaterials (such as carbon nanotubes, nanoparticles, nanowires, and more recently graphene) have been used to modify electrochemical electrodes.^{35,36} Due to their unique structural, electrical, and chemical properties, these nanoscale materials bring new prospects and previously unattainable sensitivity to electrochemical

detection by offering new sensing mechanisms, providing large surface area for detection or enzyme attachment, mediating efficient electron transfer, facilitating electrochemical reaction, and amplifying the signal of recognition events. For example, a glucose sensor developed by Kwon et al. used nanoporous and gold nanoparticle embedded carbon nanotube film as the electrode.³⁷ A remarkable sensitivity of $24.5 \mu\text{A}/\text{mM}$ was achieved due to high loading of glucose oxidase and expedited electron transfer because of interconnected conductive nanotubes and intercalated gold nanoparticles. Hou et al. demonstrated that Nafion–graphene coated electrodes exhibited higher sensitivity in dopamine detection, presumably owing to the supremely large surface-to-volume ratio and conductivity of graphene, a two-dimensional and single-atom-thick carbon sheet.³⁸

■ MICROFABRICATED DEVICES FOR ON-CHIP ELECTROCHEMICAL MEASUREMENT

Although carbon fiber amperometry has now been routinely used in many laboratories and helped in a great deal to understand the regulated cell secretion, it has several inherent limitations: (1) due to its planar surface, CFE can only record secretion from a small fraction of the entire cell membrane and, therefore, only provide poor reflection of the whole-cell secretion; (2) mass and cost production of CFE is not available; (3) it is of low throughput because of the impossibility of parallel recording on multiple cells, requirement of bulky single-cell recording setup, and nontrivial experimental procedures. Microfabrication techniques standardized in the semiconductor industry have been sought to deal with these limitations. Lithographic microfabrication offers great flexibility to make electrode(s) in array format for parallel recording and with desired shape, size, position, and material, and low-cost mass-production is feasible. Furthermore, miniaturized fully functional lab-on-a-chip devices could be fabricated with small reaction chamber, integration of microfluidics for automated solution delivery and cell positioning, and easy interface with electronic readouts.

Cell-Sized Recording Unit. Bratten et al. demonstrated the first electroanalytical microdevice to detect cell secretion.³⁹ They

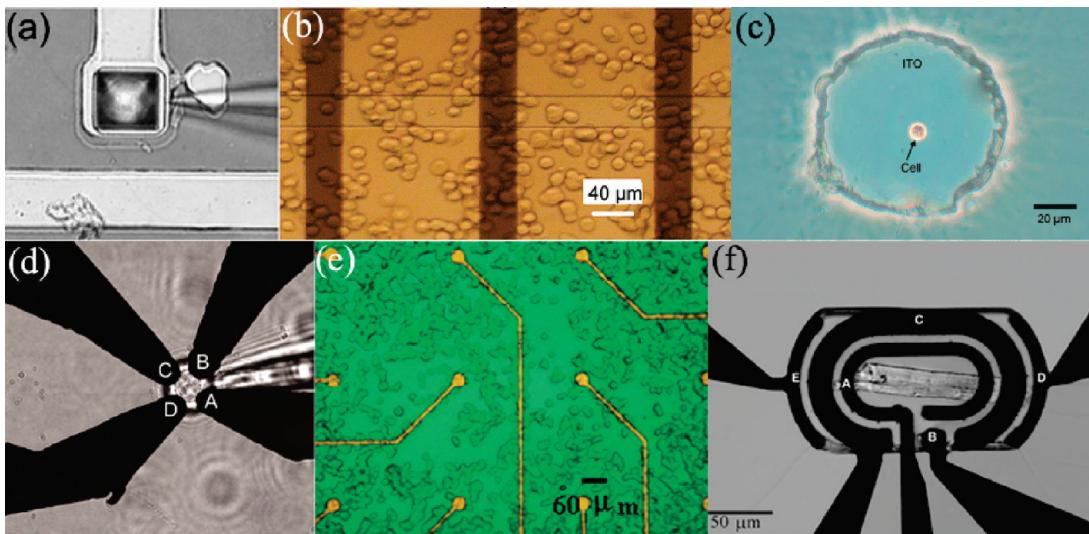


Figure 2. (a) Chromaffin cell in a well-electrode with an opening of $15\text{ }\mu\text{m}$. Adapted with permission from ref 40. Copyright 2003 American Chemical Society. (b) Chromaffin cells sitting on top of microchip using nitrogen-doped diamond-like carbon (DLC:N) microelectrodes. Adapted with permission from ref 41. Copyright 2008 Springer Science. (c) A chromaffin cell adhering to an ITO microelectrode ($100\text{ }\mu\text{m}$ diameter). Adapted with permission from ref 44. Copyright 2006 Wiley-VCH. (d) A chromaffin cell on an electrochemical detector array consisting of four platinum microelectrodes. Adapted with permission from ref 48. Copyright 2005 the National Academy of Sciences of the USA. (e) PC12 cells grown on a microelectrode array biochip (microelectrode diameter, $30\text{ }\mu\text{m}$). Adapted with permission from ref 49. Copyright 2006 American Chemical Society. (f) Micrograph of microelectrodes and a quiescent cardiomyocyte in the $20\text{ }\mu\text{m}$ deep microchamber. Adapted with permission from ref 51. Copyright 2006 the Royal Society of Chemistry.

fabricated a micrometer-scale recording chamber containing an integrated three-electrode system for enzyme-mediated electrochemical detection of purine release from single rat cardiomyocyte. The analysis is based on amperometric detection of enzymatically generated hydrogen peroxide. Because the reaction volume inside the device is only $\sim 600\text{ pL}$, femtomole of the purines (adenosine and inosine) produced by single rat cardiomyocytes could be detected. Gillis and colleagues fabricated gold microwell electrodes on a silicon chip to detect quantal release of catecholamines from bovine adrenal chromaffin cells (Figure 2a).⁴⁰ Such a picoliter-sized well electrode, which snugly houses a single chromaffin cell, is able to catch much more released catecholamines as compared to planar CFEs, thus providing a better representative measure of total cell secretion.

Electrode Materials. Electrodes with various materials could be readily deposited or sputtered on chip using commonly available fabrication equipment with low cost. The Gillis group demonstrated nitrogen-doped diamond-like carbon (DLC:N) microelectrodes on a chip to monitor quantal release of catecholamines from chromaffin cells (Figure 2b).⁴¹ The DLC:N electrode exhibits desirable electrochemical properties such as low noise, small and stable background current, and low capacitance. It is also biocompatible. Furthermore, the transparent DLC:N electrode is more advantageous than the opaque electrodes (made of, e.g., platinum, gold, or carbon) in the sense that the combination of electrochemical detection with optical imaging becomes possible. The boron-doped nanocrystalline diamond (NCD) electrode grown on a transparent sapphire wafer was recently demonstrated by another group.⁴² The indium tin oxide electrode, which has superior transparency and electrical conductivity, has also been microfabricated on chip for electrochemical detection of exocytosis.⁴³ As demonstrated by Amatore et al. (Figure 2c),⁴⁴ coupling amperometric measurement using an indium tin oxide (ITO) electrode with fluorescence imaging

could offer a comprehensive view of the “life” of a secretory vesicle before its fusion with the cell membrane and, at the same time, the kinetics of its release of molecules.

Electrode Modification. Coating of polylysine or collagen is commonly used to promote cell adhesion onto the electrodes, but such surface coating causes a decrease in amperometric signal. The oxidation products of catecholamine could passivate the electrode by forming a nonconducting polymeric film on the electrodes surface. Spégl et al. modified Au microelectrodes on a chip with mercaptopropionic acid (MPA) to detect dopamine released from PC12 cells.⁴⁵ Their results showed that modification of MPA dramatically reduced electrode passivation, improved reversibility and sensitivity, and significantly increased the storage of the transducers. Li and co-workers described a microchip with a nafion-coated carbon ink microelectrode and demonstrated that nafion coating could eliminate the interference from ascorbic acid to the measurement of catecholamine release.⁴⁶

Electrode Array. An array of individually addressable electrodes can be microfabricated on chip. The Lindau group demonstrated a four-electrode array to determine not only the release kinetics but also the exact localization of individual exocytotic events.⁴⁷ The four platinum electrodes ($3\text{ }\mu\text{m}$ in size) define the corners of a square area $\sim 10 \times 10\text{ }\mu\text{m}^2$. Catecholamines discharged from a fusing vesicle on the chromaffin cell positioned at the center of the square take different paths (thus time) to reach each electrode. Analysis of the distinct amperometric signals collected by the four electrodes allows determination of the exact location of the exocytotic site (Figure 2d). With simultaneously conducted fluorescence imaging, they confirmed that the electrochemical position assignments of single exocytotic events were well-correlated with the actual release of fluorescent vesicle markers (acridine orange).⁴⁸ Such an “electrochemical camera” provides both high spatial and high temporal

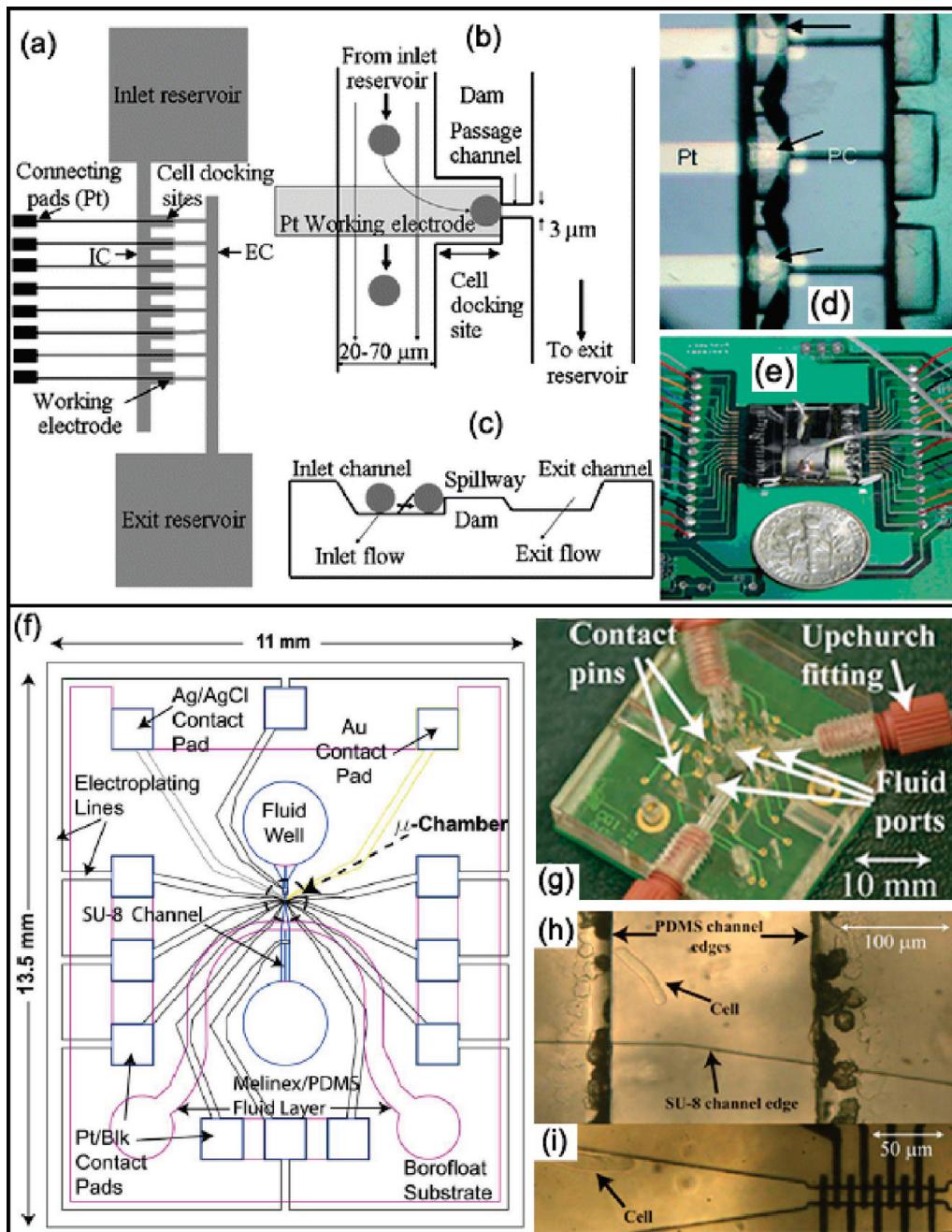


Figure 3. (a) Schematic depiction of the microfluidic cell trap device for electrochemical detection of cell secretion. IC: Inlet Channel. EC: Exit Channel. (b) An expanded view of a single docking site electrode. (c) Cross-sectional view of the device. (d) Three electrode trapping sites occupied by a single cell (top arrow) or small cell clumps (middle and bottom arrows). PC: passage channel. (e) Photo of the chip device mounted on a PCB board. a–e are adapted with permission from ref 53. Copyright 2009 The Royal Society of Chemistry. (f) Device schematic of an individual microelectromechanical system. (g) Close-up of headstage showing the electrical (pins) and fluid (ports) interfaces. (h) The OHC was selected and located by controlling flow (top to bottom) in the main channel. (i) The same cell was then pulled into the lower SU-8 fluidic layer and eventually stopped in the recording chamber. f–i are reprinted with permission from ref 54. Copyright 2008 IEEE.

information of individual exocytotic events. An electrode array also enables parallel recording on multiple cells for high throughput studies (Figure 2e)⁴⁹ or recording on selected regions in a growing cell sheet.⁵⁰ In addition, stimulation electrodes can be made in conjunction with recording electrodes to examine the stimulation-secretion coupling as shown in Figure 2f.⁵¹

Cell Targeting. Automatic and precise targeting of cells onto the electrochemical electrode or electrode array is a key challenge

for realization of genuine lab-on-a-chip devices. In the study by Spégl et al., a central aperture, concentrically surrounded by a circular sensing electrode, was fabricated on chip. It allows the cells to be captured on the aperture by suction without the need of microscope or micromanipulator.⁵² Alternatively, microfluidics has been integrated with on-chip electrochemical detection for automated handling of solutions and cells. Gillis and colleagues have demonstrated a simple reusable microchip device that

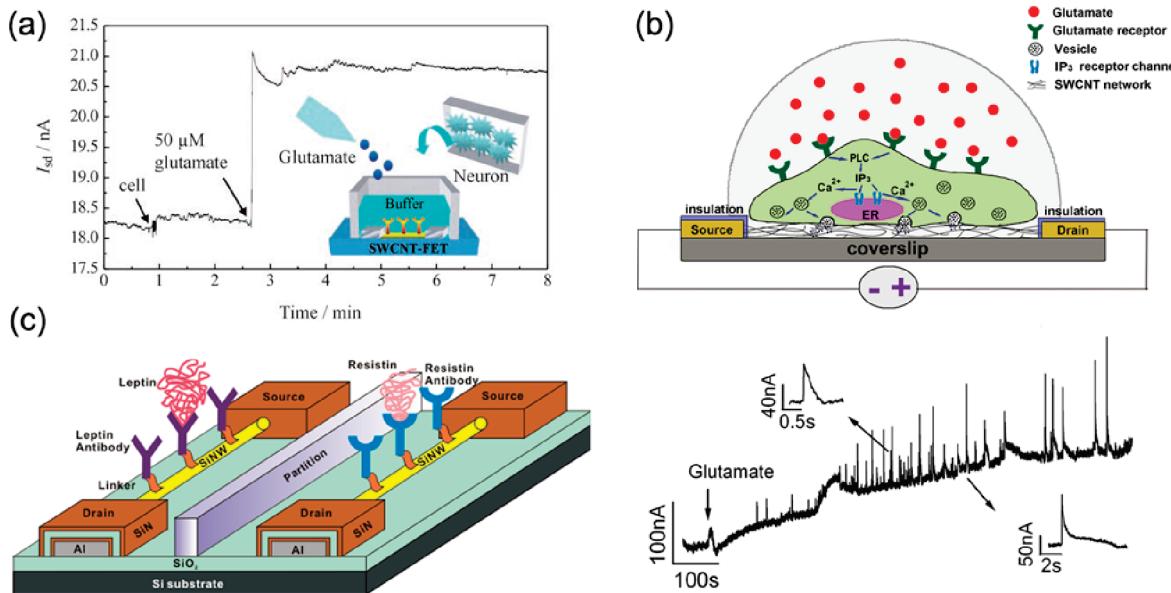


Figure 4. (a) CgA released from neurons elicited by glutamate can be detected by CgA-Ab functionalized SWCNT-FETs. Adapted with permission from ref 75. Copyright 2007 Wiley-VCH. (b) Upper: Illustration of triggered release and detection of ATP molecules (PLC: phospholipase C; ER: endoplasmic reticulum; IP₃: inositol trisphosphate); Lower: Real-time response of the SWCNT-network FET while astrocytes were stimulated by neurotransmitter glutamate. Adapted with permission from ref 77. Copyright 2009 Elsevier. (c) Schematic illustration of a SiNW chip functionalized with leptin antibodies and resistin antibodies in two separate sensing regions. Adapted with permission from ref 80. Copyright 2009 the Royal Society of Chemistry.

utilizes microfluidic traps to automatically target individual cells onto the array of recording electrodes made in register with the trap sites (narrow spillways that cells cannot pass; Figure 3a–e).⁵³ In a study by Dittami et al., multilayered microfluidics formed by SU-8 or PDMS channels was used to select a specific type of cells in a cell mixture (by size screening) and guide them to the electrode array for electrochemical detection of cell secretion together with impedance analysis of membrane properties (Figure 3f–i).⁵⁴ A drawback of the suction or microfluidic targeting of cells is that the pressure gradient needed to position the cells may cause cell damage or unintended influences on exocytosis (e.g., due to induced membrane tension). Therefore, surface chemistry and a patterning approach may be used to recruit cells to the electrode(s). Gangopadhyay and Gillis collaboratively developed a simple approach in which Teflon AF film was used to insulate an array of conductive DLC:N line patterns and resist cell attachment; then, cell-sized holes were etched through the Teflon film to define the working area of the DLC:N electrode and cell docking sites.⁵⁵ Cells could self-register onto the exposed electrode surface because DLC:N film is cytophilic.

Stimulation. Commonly, cell exocytosis is stimulated by delivering depolarizing solutions (e.g., a solution with elevated potassium) using a separate fluidic infrastructure. This complicates the chip design and causes time delay between the stimulation and the actual cell response because of fluid transport and exchange. To avoid the problems associated with solution delivery, photolysis of caged Ca^{2+} was used to stimulate chromaffin cells on chip with a uniform and rapid rise of $[\text{Ca}^{2+}]_i$ inside the target cell.⁵⁶ This is desirable to investigate the fast stimulation-secretion coupling and the Ca^{2+} dependence of this process. However, this approach requires preloading the cells with membrane permeable cage compounds (ester form of nitrophenyl-ethylene glycol bis(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA)). Recently, Dittami and Rabbitt presented a

microchip, in which the cells were trapped over the working electrodes by a 5 μm constriction in the fluidic channel, and two electrodes on the two sides of the blocked channel were used to deliver a voltage pulse across the cell to electrically evoke exocytosis.⁵⁷

Current Issues. Although the techniques highlighted in this section promise high-throughput lab-on-a-chip analysis of cell secretion, they inherit all the limitations of electrochemical detection. Nevertheless, as discussed earlier, various strategies may be used to relieve these limitations. Undoubtedly, the full potentials of on-chip electrochemical detection are still far to be reached. Since fabrication of microchips is not easily accessible or affordable to most laboratories, these techniques would not become routine low-cost laboratory tools unless mass-produced commercial products become available. Therefore, efforts toward practical applications and commercialization are highly desired.

■ MEMBRANE CAPACITANCE MEASUREMENT BASED ON PATCH CLAMPING RECORDING

The Nobel Prize winning technique, patch clamp, best exemplifies how miniaturized probes allow microscopic or nanoscopic investigation on cell functions. In this technique, a glass pipet electrode with a tip size of 1–2 μm is used to resolve ionic current from a single or a few protein ion channels on the recorded cell membrane patch (patch mode) or from many ion channels on the entire cell membrane (whole-cell mode). Exocytosis can be electrically detected using membrane capacitance measurement based on patch clamping recording, because incorporation of vesicle membrane with the plasma membrane during exocytosis leads to an increase in cell surface area (thus capacitance of the cell membrane).^{58,59} Fusion of a large secretory vesicle can cause a steplike increase in cell membrane

capacitance,⁶⁰ and the exocytotic kinetics of a cell can be determined with millisecond resolution.

However, patch clamp (thus membrane capacitance measurement) requires highly skilled operators and is of extremely low throughput. Typically, only a few successful recordings can be made per day. Microfabrication has been employed to replace the glass micropipet with a planar aperture on chip. Patch clamp-on-a-chip has been demonstrated by several groups,^{61–63} and automated patch clamp systems with multiplexed recording electronics and sophisticated microfluidics that enables high-throughput recording have already been commercialized.^{64,65} This greatly interests the pharmaceutical companies who have millions of potential drug leads targeting on ion channels to be screened. In principle, patch clamp-on-a-chip can be utilized for automated membrane capacitance measurement and high-throughput drug screening targeting on exocytosis.

In comparison with electrochemical detection, membrane capacitance measurement can reveal the exocytotic kinetic from a membrane patch (on-cell-patch mode) or from the entire cell (whole-cell mode) and can be used for vesicular exocytosis of nonoxidizable substances, but it only provides indirect measurement that could be interfered by the endocytosis process and the accompanying large transmembrane ionic current.⁶⁶ Simultaneous electrochemical detection and membrane capacitance measurement have been employed to utilize the merits of both approaches for more in-depth investigations.^{67,68} The Lindau group investigated exocytosis of individual chromaffin granules using cell-attached membrane capacitance measurements combined with electrochemical detection of catecholamines.⁶⁹ It was achieved by inserting a fine carbon-fiber electrode into a patch pipet. This so-called patch-amperometry technique allows simultaneous and precise determination of the vesicle size, quantal content, fusion pore conductance, and permeability of the discharged compounds through the fusion pore.⁷⁰

■ NANO ELECTRONIC SENSING

The recently emerging nanoelectronic biosensing based on nanostructured semiconducting materials (e.g., carbon nanotubes and silicon nanowires) opens a novel avenue to electrically detect dynamic cellular activities,^{71,72} such as cellular bioelectricity (the usual trigger of exocytosis in excitable cells)^{73,74} and secretion of biomolecules (as discussed below). Because their current flow solely or largely on the surface, the conductance of semiconducting nanomaterials is highly sensitive to minute electrochemical perturbations induced by the interacting biomolecules.

Carbon Nanotube Based Transistors. In the work of Chen and colleagues,⁷⁵ field-effect transistors (FETs) with one single-walled carbon nanotube (SWCNT) as the conducting (sensing) channel were employed to detect glutamate-triggered vesicular release of chromogranin A (CgA, a marker for neuroendocrine cancer) from a population of living cortical neurons, with a detection limit of ~ 1 nM (Figure 4a). The specificity of detection was achieved by functionalizing the recognition elements, goat immunoglobulin G antibodies against CgA, onto the SWCNT. The binding of CgA to the antibodies on the SWCNT caused increase in SWCNT conductance (or current) due to electrostatic gating effect (field-effect) introduced by the negatively charged CgA molecules. Using the same device, histamine-triggered release of CgA from a single chromaffin cell positioned right above the SWCNT was detected with an estimated sensitivity as low as \sim fM.⁷⁶

Fabrication of single-SWCNT FETs is, however, nontrivial due to the challenges associated with microfabrication and assembly of SWCNT onto the electrodes. We have developed SWCNT-network FETs readily fabricated on the benchtop, which provide a larger sensing area and can serve as the cell growth substrate to directly interface with a few hundred of cells. Using such devices, the vesicular release of adenosine triphosphate (ATP) molecules from astrocytes triggered by glutamate (Figure 4b)⁷⁷ and catecholamine release from PC12 cells triggered by membrane depolarization⁷⁸ were detected with millisecond resolution. ATP and catecholamine molecules discharged into the nanogap between the cell membrane and the SWCNTs intimately interact with SWCNTs via pi-pi interaction and consequently alter the SWCNT conductance through electrostatic gating or doping effects, respectively. Single cell secretion of these molecules led to distinct current spike response of the SWCNT-network FET. In these applications, the specificity of detection is ensured in the well-defined biological context (stimulation-secretion coupling).

Silicon Nanowire Based Transistors. We have also fabricated nanoelectronic chips with array of silicon nanowires (SiNWs) using top-down complementary metal oxide semiconductor (CMOS) compatible methods standardized in the semiconductor industry (therefore, mass and low-cost production is possible).⁷⁹ By functionalizing SiNWs with specific antibodies as the recognition elements, the secretion dynamics of hormone leptin and resistin from adipocytes were resolved simultaneously on one chip with high specificity and femtomolar sensitivity (Figure 4c).⁸⁰ Recently, similar devices were used to specifically and parallelly detect dynamic secretion of proinflammatory cytokines (tumor necrosis factor (TNF)-alpha and IL6) from macrophages in response to the stimulation of bacterial endotoxin.⁸¹ In addition, the sensor's ability to obtain faithful measurement in complex biological fluids allowed us to reveal the transient elevation of these cytokines in blood during rat's immune response to intraperitoneally injected endotoxin. In principle, hundreds of individually addressable nanowires enable simultaneous detection of many signaling molecules complicated in a particular biological reaction (e.g., immune response). It is extremely useful to study, diagnosis, or drug assessment for complex physiological phenomena from systems point of view, and it has recently been demonstrated that the sensitivity of SiNW FETs can be further improved by incorporating on-chip electrokinetic enrichment of the target molecules near the sensing region.⁸² Using two metal electrodes fabricated besides the SiNW, the molecules were transported and concentrated by the dielectrophoretic force arising from the applied nonuniform AC electric field.

Advantages and Limitations. A nanoelectronic approach, with a simple detection scheme, provides high temporal resolution, amenability of lab-on-a-chip devices, and possibility of parallel detection with an array of sensing elements. In addition, the nanoscale sensing elements could offer high spatial resolution, so that localized detection at a subcellular domain can be realized, and the array format of the sensors may allow spatially resolved detection of signal propagation⁷⁴ and hot spots. In addition to carbon nanotubes and silicon nanowires as highlighted here, other nanostructured semiconducting materials have also been used as the active channel in a transistor and demonstrated their potentials in biomolecular detection, including ZnO nanowire,⁸³ In₂O₃ nanowire,⁸⁴ and recently graphene.^{85,86} In particular, graphene (the flat cousin of carbon nanotubes) is emerging as the unique or better alternative to carbon nanotubes and other currently used one-dimensional

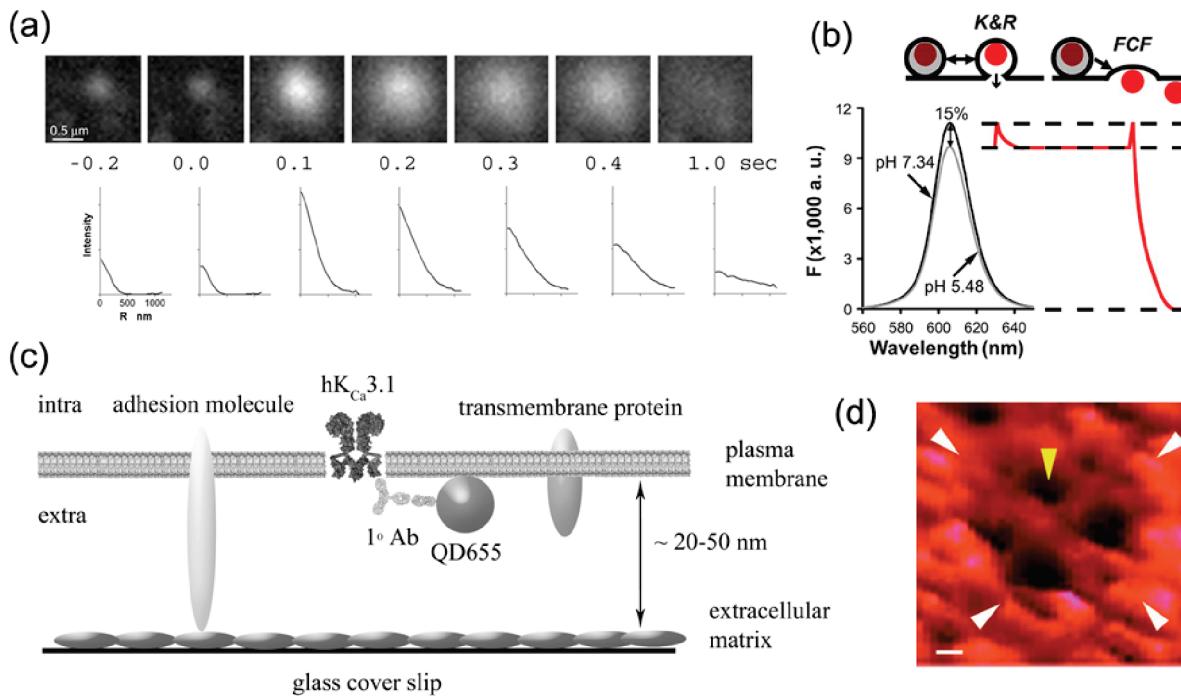


Figure 5. (a) Fusion event of a Vamp-GFP-labeled vesicle revealed by TIRFM. Adapted with permission from ref 91. Copyright 2008 John Wiley and Sons. (b) Distinguish kiss-and-run (K and R) and full-collapse fusion (FCF) based on pH-dependent photoluminescence of the quantum dot loaded into the synaptic vesicle. Adapted with permission from ref 103. Copyright 2009 Science. (c) Labeling of human potassium channel (hK_{Ca}3.1) in the plasma membrane of migrating Madin-Darby canine kidney focus (MDCK-F) cells with quantum dots. Adapted with permission from ref 104. Copyright 2008 the American Physiological Society. (d) AFM micrographs revealing the structure of the fusion pore at the cell plasma membrane in exocrine and neuroendocrine cells. A pit (white arrowheads) and four depressions (yellow arrowhead) are identified in a live pancreatic acinar cell. Scale bars = 100 nm. Adapted with permission from ref 109. Copyright 2006 Elsevier.

materials in biosensing because of its extraordinary structural, electrical, and physiochemical properties.⁸⁷ Recently, we have used FETs based on microfluidic-patterned thin-film of chemically converted graphene to detect catecholamine secretion from PC12 cells.⁸⁸ The expanding variety of available building blocks for nanoelectronics provides rich possibilities in device configuration, sensing mechanism, surface chemistry, and so on.

On the other hand, although nanoelectronic biosensing provides a versatile platform for many possible applications, it appears that the detection can only be made when the target molecules or biological processes are able to directly introduce the following scenarios: change of the local dielectric or pH environment, electrostatic gating imposed by the charges on the interacting molecules, or charge transfer between the interacting molecules and the sensing nanomaterial (doping effect). In an effort to extend the detection range of nanoelectronic biosensors, Stern et al. developed a hybrid nanoelectronic enzyme-linked immunosorbent assay (ne-ELISA) that combines the power of enzymatic conversion of a bound substrate with electronic detection.⁸⁹ In contrast to colorimetric measurement in ELISA, protein binding (here, interleukin-2) was quantitatively detected on the basis of enzymatic reaction induced pH increase, which, in turn, caused conductance change of In₂O₃ nanowire.

IMAGING SECRETORY VESICLES AND EXOCYTOSIS

Direct imaging of secretory vesicles before and upon fusion is obviously instrumental to reveal the molecular events in the exocytotic cascade. The unambiguous evidence of synaptic vesicles and their fusion at synaptic membrane was provided

by transmission electron microscopy three decades ago, and confocal microscopy has now widely been used to reveal the distribution of fluorescently labeled secretory vesicles inside a cell. In this section, we briefly discuss several new techniques applied to visualize exocytotic process at nanoscale and in real-time.

Total Internal Reflection Fluorescence Microscopy (TIRFM). Total internal reflection fluorescence microscopy (TIRFM), which evanescently illuminates the thin section (<200 nm) just above the interface between the glass coverslip and the adherent cell membrane, is a powerful tool to visualize the molecular events occurring immediately adjacent to or at the cell membrane with high temporal resolution as compared with confocal microscopy and with minimal interference from the background signals (e.g., autofluorescence from the cell body). It complements the electrochemical⁹⁰ and electrophysiological measurements.⁹¹ The presence, trafficking, and final fusion of individual fluorescently labeled secretory vesicles in the subplasmalemmal region can be resolved under TIRFM (Figure 5a).^{91,92} It has been revealed by TIRFM that vesicles undertake constant lateral movement (parallel to the cell membrane) and vertical movement (transition between cytosol and subplasmalemmal region), and the vesicle trafficking is positively correlated with the vesicle fusion competence and whole-cell secretion kinetics.^{93–95} The widely presumed notion of a long-lived docked state as a prelude to fusion has been refuted by the TIRFM observation that multiple tethering states and increased vesicle travel immediately precedes the fusion event.⁹⁶

Super-Resolution Fluorescence Imaging. The resolution of optical imaging is fundamentally limited by diffraction (~100 nm). This seemingly insurmountable barrier of diffraction limit has

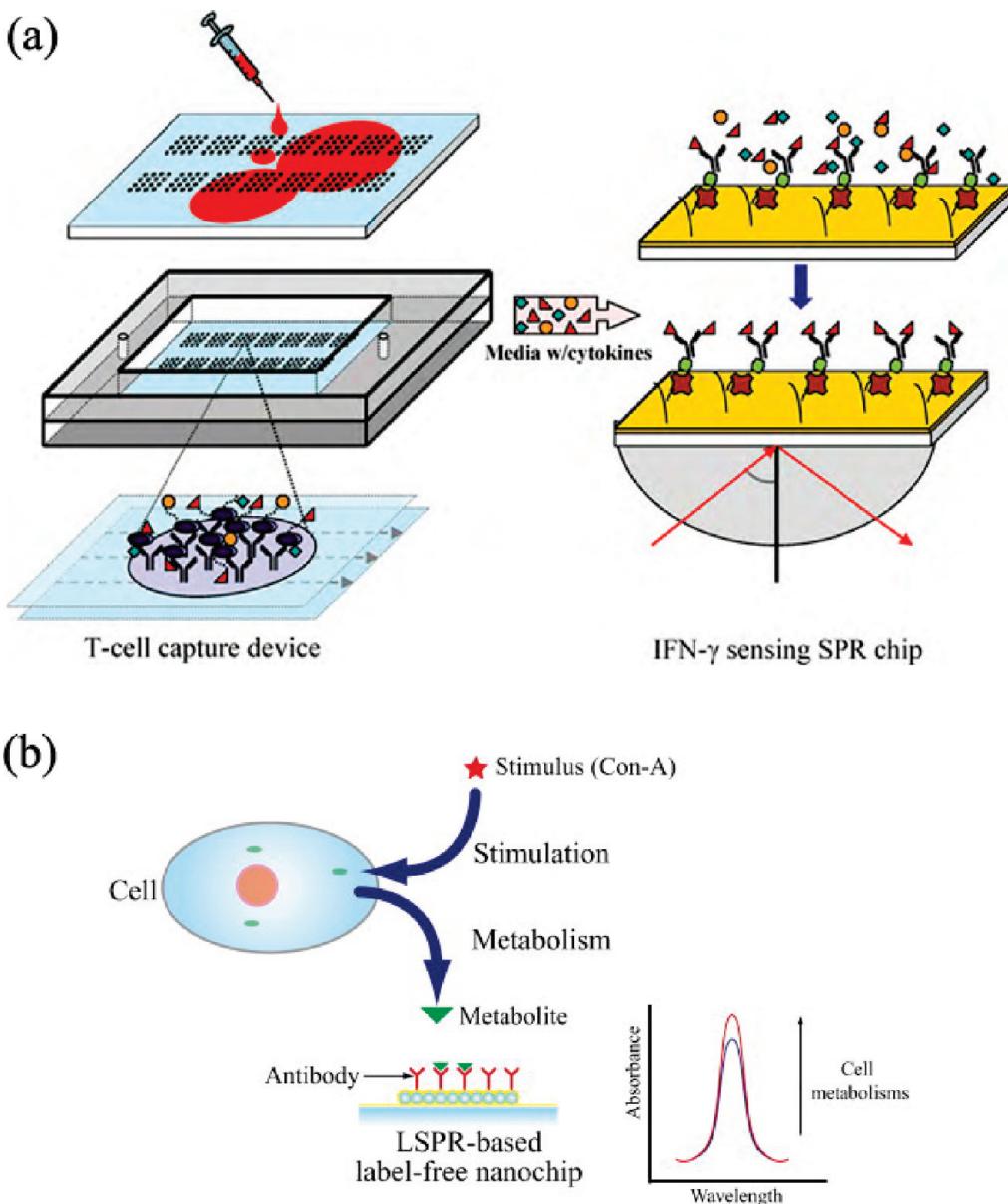


Figure 6. (a) Detecting interferon- γ secretion from CD4 T-cells using a SPR device. Left: Glass slides imprinted with anti-CD4 antibodies spots are enclosed inside a flow chamber and exposed to RBC-depleted human blood. Right: After affinity selection, CD4 T-cells are activated *in situ* to secrete cytokines. Media bathing T-cells is then sent into an SPR chip containing an antibody layer for detection of IFN- γ . Adapted with permission from ref 115. Copyright 2010 Elsevier. (b) Illustration of LSPR detection of stimulated cell secretion (in particularly, interleukin-2 secretion in this study). Adapted with permission from ref 118. Copyright 2008 Elsevier.

recently been broken by several novel techniques, namely, stimulated emission depletion (STED) microscopy, saturated structured illumination microscopy (SSIM), stochastic optical reconstruction microscopy (STORM), and photoactivated localization microscopy (PALM).⁹⁷ All these methods have already demonstrated their potential to visualize biochemical processes in live cells with molecular-scale resolution.

In particular, a STED microscope with a spatial resolution of 45 nm was devised to resolve individual synaptic vesicles within nerve terminals.⁹⁸ It was revealed that synaptotagmin (the Ca^{2+} sensor for vesicular exocytosis) remains clustered after exocytosis. Later, the same group used STED to track synaptic vesicle movement within the highly confined space of synaptic boutons.⁹⁹ In the work by Sieber et al.,¹⁰⁰ STED microscopy

was used to resolve individual clusters of syntaxin 1 on cell membrane (a member of SNARE proteins implicated in vesicle fusion and docking). It was discovered that the syntaxin cluster has a size of 50–60 nm and contains ~90 syntaxin molecules. In brief, the extraordinary (actually, theoretically unlimited) resolving power of STED is achieved by applying patterned illumination to differentially modulate the emission ability of the fluorophores within a diffraction-limited region. We foresee that, with the application and advance of these revolutionary super-resolution fluorescence imaging techniques, many previously unattainable questions on the exocytotic process would soon be addressed, for example, to determine the size, lifetime and dynamics of membrane domains (lipid rafts) that mediate exocytosis.

Quantum Dots. Conventionally, organic fluorophores are used to label membrane proteins and secretory vesicles. However, the organic fluorophores subject to several inherent problems including serious photobleaching and usually weak fluorescence. Photoluminescent quantum dots (QDs) are emerging as the alternative fluorophores with unique advantages such as high brightness, high photostability (therefore virtually immune to photobleaching), broad adsorption, and narrow and size tunable emission. Using QDs, long-term tracking and multicolor labeling under single excitation wavelength in live cells become possible.^{101,102}

In a recent report, Zhang et al. have labeled synaptic vesicles in hippocampal neurons with QDs using weak-stimulation facilitated endocytotic loading.¹⁰³ Taking advantage of the pH-dependent photoluminescence of QDs, they were able to distinguish kiss-and-run from full-collapse fusion because kiss-and-run (partial fusion) allows protons but not the QDs to escape from the vesicle, consequently, resulting in transient deacidification of the vesicle and QD brightening (Figure 5b). Owing to the photostability of QDs, they could track single vesicles through multiple rounds of kiss-and-run and reuse without disturbing the vesicle cycling. In the study by Nechyporuk-Zloy et al., functionalized QDs have been used to label K⁺ channels that play a role in stimulation-secretion coupling by conjugating QDs with antibodies against K⁺ channels (Figure 5c).¹⁰⁴ The diffusion kinetics of individual QD tagged K⁺ channels were, for the first time, revealed under TIRFM. Functionalized QDs have also been employed to investigate the glycosphingolipid GM1 enriched membrane domains (lipid rafts) which are thought to play essential roles in vesicle docking and fusion.¹⁰⁵

Besides QDs, other nanoparticles (NPs) have also been used for molecular and cellular imaging, including metallic (Au and Ag) NPs,¹⁰⁶ lanthanide NPs,¹⁰⁷ and silica NPs.¹⁰⁸ In view of the problems of poor solubility and fluorescence intermittency associated with QDs, Beaurepaire et al. proposed lanthanide ion-doped oxide nanoparticles as a promising new class of biological fluorescent labels.¹⁰⁷ They have used them to specifically label Na⁺ channels that are critically involved in stimulation-secretion coupling in excitable cells.

Scanning Probe Microscopy. In complement with optical microscopy, scanning probe microscopy using ultramicroelectrodes or nanoprobes has been applied to investigate exocytosis with high spatial resolution. The fusion pore structures formed between the vesicular membrane and the plasma membrane have been revealed by atomic force microscopy (AFM) on live cells at near nm resolution and in real-time (Figure 5d).¹⁰⁹ The fusion structures have also been identified by scanning ion conductance microscopy (SICM).¹¹⁰ Scanning electrochemical microscopy (SECM) invented by Bard and colleagues in the late 1980s provides the possibility for spatially resolved real-time monitoring of molecular secretion from single cells and, at the same time, providing the topographic information of the cell surface.¹¹¹ Detection of dynamic release of signaling molecules such as catecholamines,¹¹² nitric oxide,¹¹³ and alkaline phosphatase¹¹⁴ from living cells has been demonstrated by SECM.

SURFACE PLASMON RESONANCE

Surface plasmon resonance (SPR) is a sensitive tool to detect the adsorption of biomolecules onto an antibody functionalized planar surface based on changes in the refractory index. In the system developed by Stybayeva et al.,¹¹⁵ a flow chamber upstream of a SPR detector was microprinted with CD4 antibodies

to capture CD4 T-cells from human blood, and interferon- γ (IFN- γ) released by the captured cells was then detected by routing effluent from the fluidic chamber onto an SPR chip modified with IFN- γ antibodies (Figure 6a). Chou et al. demonstrated that the SPR biosensor is able to quantify interleukin-6 secretion from human fibroblast MRC5-CVI cells triggered by lipopolysaccharide, without any prior sample preparation procedures such as centrifugation or dilution.¹¹⁶ In a recent noteworthy work, Anderson et al. presented a SPR biosensor to monitor the release of troponin T (TnT) from cardiomyocytes and assess drug-induced cardiotoxicity with a detection limit of 10 ng/mL.¹¹⁷

In contrast to conventional SPR sensors based on flat metal films, localized surface plasmon resonance (LSPR), which uses nanometer-sized metallic structures, provides even higher sensitivity and can be excited without any restrictions on the angle of incidence. In a demonstration by Endo et al.,¹¹⁸ LSPR sensors were constructed by coating silica nanoparticles with gold followed by functionalization of antibodies against interleukin-2 (IL-2). They were used for label-free detection of IL-2 release from mouse thymus cells in response to concanavalin-A stimulation, as signaled by the increment in the absorbance intensity (Figure 6b). Also noteworthy is a compact nanoplasmonic biosensor chip with integrated electrical detection, recently demonstrated by Mazzotta et al.¹¹⁹

Although also relying on specific immunoreaction between the antibodies and the targets, as compared with biochemical assays, SPR detection provides higher throughput, higher sensitivity, small sample consumption, and possibility of lab-on-a-chip analysis. However, unlike electrochemical or electrical detection, SPR is not able to provide millisecond resolution to resolve rapid exocytosis.

MOLECULAR SENSING ELEMENTS

Although here the emphasis is placed on the micro- or nanofabricated biosensors or probes, in this section, we briefly review a few examples, in which the sensing element is the natural or genetically engineered bionanodevices. When these molecular sensing elements are attached to a cell, local detection can be made on the cell surface. When these molecular sensing elements are expressed in the target cell or the reporter cell, they function in their native environment, and the signal can be amplified due to their abundant expression or intracellular reaction cascade through built-in molecular machineries inside the cell.^{120–123}

The firefly luciferase assay has widely been used to detect the presence of ATP molecules in bulk solution. It, however, does not reflect the dynamic ATP flux at the cell membrane. Nakamura et al. immobilized biotinylated luciferase onto the biotinylated cell surface (conjugation mediated by streptavidin) in order to detect local ATP release from human pulmonary artery endothelial cells.¹²⁴ ATP secretion from astrocytes has also been indirectly detected using a so-called sniffer cell approach.¹²⁵ In this study, HEK-293T cells were overexpressed with the ATP-binding purinergic P2 × 3 receptors and plated onto the cultured cortical astrocytes. HEK-293T cells were then patch clamped to record ATP-triggered membrane currents while the neighboring astrocytes were stimulated. Using this approach, the dynamics of ATP release from single astrocytes was reported.

Fluorescence resonance energy transfer (FRET) refers to the phenomenon in which a bioluminescence or chemiluminescence donor molecule transfers nonradiative energy to a nearby

luminescent acceptor molecule. FRET occurs efficiently only when the donor and the acceptor are in close proximity (1–10 nm), a distance that protein–protein interaction occurs. FRET has been used to detect glutamate secretion from neurons.¹²⁶ The fluorescent indicator protein for glutamate (FLIPE) was constructed by fusing the glutamate binding protein from *Escherichia coli* with two variants of the green fluorescent protein (as the FRET partners). In the presence of glutamate, FRET efficiency decreased in a concentration-dependent manner. When FLIPEs were expressed in rat hippocampal neurons, depolarization of neurons leads to secretion of glutamate and a reduction in FRET efficiency corresponding to 300 nM glutamate at the cell surface. In the recent work by Nguyen et al.,¹²⁷ HEK293 cells stably expressing the M1 receptor (a major muscarinic receptor in neocortex) and the FRET fusion protein TN-XXL (a Ca^{2+} indicator) were used as the reporter cells to detect acetylcholine release from the frontal cortex of the adult rat. Activation of the M1 receptor upon acetylcholine binding increased cytosolic calcium in HEK293 cells via the Gq/inositol triphosphate (IP_3) second messenger pathway. The subsequent binding of Ca^{2+} to TN-XXL induces a conformational change that enhances FRET between its cyan and yellow fluorescent protein domains.

Molecular biosensing elements are designed and perfected by nature through evolution. They provide high sensitivity and high specificity, and owing to their small size, highly localized detection may be realized. On the other hand, development and implementation of such biological sensing systems are often nontrivial and of low throughput due to involvement of molecular and cellular engineering and challenging recording procedures.

■ OTHER TECHNIQUES AND POSSIBILITIES FOR INTRACELLULAR INTERROGATIONS

The application of micro- and nanotechnologies to the study of cell secretion is only limited by imagination. Besides those highlighted here, many other micro/nanotools or technologies could also be borrowed to investigate the process of cell secretion. For instance, we have demonstrated that micropatterning and surface functionalization could be used to immobilize and pattern molecular probes targeting on membrane lipids or proteins relevant to secretion, in order to examine the spatially defined regulations by organized functional membrane domains.¹²⁸ Nanolithography, nanopatterning, or nanoprinting could similarly be used to study the exocytotic process and its regulation at molecular scale. As another instance,¹²⁹ nano-optical-fiber immobilized with lactate dehydrogenases, which converts lactate into pyruvate and the fluorescent byproduct reduced nicotinamide adenine dinucleotide (NADH), have been used by Zheng et al. to locally detect lactate efflux at the cell surface.

In addition to the provision of high spatiotemporal resolution and high sensitivity, the nanoprobe, which could be inserted inside the cell with minimal damages, may be used for intracellular interrogations to unravel information on, such as, synthesis, concentration, compartmentalization, and transport of the secretory compound. For example, a membrane impaling electrochemical nanoelectrode can be used to map the concentration profile of an intracellular molecule.¹³⁰ Singhal et al. recently reported a remarkable cellular endoscope conducted with a carbon nanotube mounted at the tip of a microglass pipet. It

can be used to transport fluids into a cell and perform optical (surface enhanced Raman spectroscopy) and electrochemical diagnostics at the single organelle level.¹³¹ Potentially, this technique could be used for intravesicular interrogations. Also recently, Lieber and colleagues have created a silicon nanowire transistor so small (smaller than many viruses or intracellular organelles) to penetrate cell membrane and record intracellular electrical signal.¹³² In principle, such nanoFET may also be used to detect intracellular molecules and exocytotic steps occurring at or near the cell membrane. With the rapidly expanding arsenal of nanomaterials and micro/nanofabrication/manipulation tools, it should be expected that novel techniques to study the spatio-temporally regulated exocytosis will continue to emerge.

■ PERSPECTIVES

In this article, we have reviewed a number of techniques based on different detection modalities and discussed their advantages and disadvantages. As cell secretion is such a complex process and many questions remain to be answered; there is no omnipotent approach which is ideal to study all aspects of the exocytotic process. Instead, the choice of the method should depend on the specific study and requirements with balanced considerations. We have introduced several examples of integrating different detection principles, specifically, combination of electrochemical and optical measurements,⁴⁴ combination of electrochemical and electrical measurements,^{69,70} and combination of plasmonic and electrical detection.¹¹⁹ Obviously, a device equipped with multiple sensing capabilities would provide richer information, higher throughput, and better fidelity. Such integrative devices or sensors are highly anticipated.

The new micro- and nanotechnologies are beginning to provide fundamental insights into an exocytotic process at the cellular and molecular level that were previously unattainable. We look forward, in particular, to seeing more novel nanotechnologies that will allow us to directly and closely investigate the neurotransmitter secretion and its complex regulations at synaptic terminals, which are only tens of nanometers in diameter and packed with hundreds of protein species and tiny synaptic vesicles. This is a holey grail in neuroscience technology.

Secretion is an essential cell function, malfunction of which leads to various critical diseases. Some drugs that interfere with the exocytotic process have been used clinically to treat different diseases. For example, L-dopa (a dopamine precursor) can relieve the Parkinson's symptom by increasing the amount of dopamine packed into the secretory vesicles; botulinum toxins, which inhibit the exocytosis by disrupting the SNARE complex and fusion pore structure, can be used to treat cervical dystonia.¹³³ The discovery and exploitation of secretion targeting drugs, however, have been largely hindered by the lack of routine high-throughput techniques to probe cell secretion. Therefore, it is of paramount importance to develop functional devices for high-through drug screening targeting on cell secretion. Cui et al. recently provided the proof-of-concept demonstration of the use of microchip devices to readily assess the effects of drugs (L-dopa, reserpine (vesicular monoamine transporter), and nomifensine (a dopamine transporter inhibitor)) on dopamine secretion from PC12 cells.⁴⁹

With the rapid advance of micro/nanotechnologies, we envision that integrated and miniaturized lab-on-a-chip devices would soon be commercially available for high-throughput functional studies, diagnosis, and drug screening by transforming

the specialists' niches to readily available benchtop tools for biologists and pharmaceutical companies. Since cell secretion intimately couples with many other cell functions (e.g., ion channel activities), the high-throughput platforms for the cell secretion assay would also be useful to indirectly probe other cell functions and to assess drugs targeting on those functions. We hope this Review would inspire researchers in different fields to develop interdisciplinary solutions for the study and drug discovery of cell secretion.

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BIOGRAPHIES

Yinxia Huang studied biochemistry at the Nanjing University (China), where she received her bachelor and Ph.D. degrees in 2003 and 2007, respectively. Since 2007, she worked as a postdoctoral researcher in the School of Chemical and Biomedical Engineering at Nanyang Technological University (Singapore). Her current research activities focus on the development of nanoelectronic biosensors for detection of biomolecules and cellular activities, based on carbon nanotube and graphene.

Dong Cai studied biomedical engineering at Huazhong University of Science and Technology (China), where he received his Diploma and Ph.D. in 1998. He worked as a postdoctoral researcher in the Department of Physiology at the University of Missouri–Columbia between 1998 and 1999 and then the Department of Neuroscience at Tufts University from 1999 to 2003 to study biophysics of exocytosis and Ca-channel modulation. During 2003 to 2006, he was a senior scientist in Nanolab, Inc (USA), a start-up company of nanotechnology and nanomaterials, where he was in charge of biotechnology developed with nanomaterials. In 2006, he became Research Associate Professor at Boston College, Department of Biology. His latest research is focused on molecular imprint based biorecognition of proteins, biosensor array to detect cancer biomarkers and viral antigens, and biointerface for neuronal activity studies.

Peng Chen received his bachelor and master degrees from Zhejiang University (China) in 1992 and 1994. He then studied in the University of Missouri–Columbia, where he received his Ph.D. in 2002 and conducted research on exocytosis and electrophysiology. This was followed by a period of postdoctoral research at Harvard University, where he worked on nanopore sensing technology. In 2005, he joined the Division of Bioengineering in the School of Chemical and Biomedical Engineering at Nanyang Technological University (Singapore) as an Assistant Professor. He is now an Associate Professor in the same division. His current research focuses include nanoelectronics, nanoelectronic biosensing, bionanotechnology, and exocytosis. He is particularly interested in applying interdisciplinary and integrative approaches to study biomedical problems.

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REFERENCES

- (1) Ivanov, A. I. *Methods in Molecular Biology*; Humana Press: Totowa, NJ 2007; Vol. 440.
- (2) Sollner, T. H. *Mol. Membr. Biol.* **2003**, *20*, 209–220.
- (3) Salaun, C.; James, D. J.; Chamberlain, L. H. *Traffic* **2004**, *5*, 255–264.
- (4) Burgoyne, R. D.; Morgan, A. *Physiol. Rev.* **2003**, *83*, 581–632.
- (5) Martin, T. F. J. *Trends Cell Biol.* **1997**, *7*, 271–276.
- (6) Yang, Y.; Udayasankar, S.; Dunning, J.; Chen, P.; Gillis, K. D. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 17060–17065.
- (7) Gillis, K. D.; Chow, R. H. *Semin. Cell Dev. Biol.* **1997**, *8*, 133–140.
- (8) Xue, R. H.; Zhao, Y. Y.; Chen, P. *Biochem. Biophys. Res. Commun.* **2009**, *380*, 371–376.
- (9) Lang, T.; Bruns, D.; Wenzel, D.; Riedel, D.; Holroyd, P.; Thiele, C.; Jahn, R. *EMBO J.* **2001**, *20*, 2202–2213.
- (10) Kurien, B. T.; Scofield, R. H. *Methods* **2006**, *38*, 283–293.
- (11) Popii, V.; Baumann, G. *Clin. Chim. Acta* **2004**, *350*, 1–16.
- (12) Nguyen, D. N.; Becker, G. W.; Riggan, R. M. *J. Chromatogr. A* **1995**, *705*, 21–45.
- (13) Pan, W. Y.; Chen, W.; Jiang, X. Y. *Anal. Chem.* **2010**, *82*, 3974–3976.
- (14) Koster, S.; Angile, F. E.; Duan, H.; Agresti, J. J.; Wintner, A.; Schmitz, C.; Rowat, A. C.; Merten, C. A.; Pisignano, D.; Griffiths, A. D.; Weitz, D. A. *Lab Chip* **2008**, *8*, 1110–1115.
- (15) Sun, S.; Yang, M. H.; Kostov, Y.; Rasooly, A. *Lab Chip* **2010**, *10*, 2093–2100.
- (16) Liu, Y.; Wang, H. X.; Huang, J. Y.; Yang, J.; Liu, B. H.; Yang, P. Y. *Anal. Chim. Acta* **2009**, *650*, 77–82.
- (17) Tsuyama, N.; Mizuno, H.; Tokunaga, E.; Masujima, T. *Anal. Sci.* **2008**, *24*, 559–561.
- (18) Mizuno, H.; Tsuyama, N.; Harada, T.; Masujima, T. *J. Mass Spectrom.* **2008**, *43*, 1692–1700.
- (19) Leszczyszyn, D. J.; Jankowski, J. A.; Viveros, O. H.; Diliberto, E. J.; Near, J. A.; Wightman, R. M. *J. Biol. Chem.* **1990**, *265*, 14736–14737.
- (20) Schulte, A.; Schuhmann, W. *Angew. Chem., Int. Ed.* **2007**, *46*, 8760–8777.
- (21) Kita, J. M.; Wightman, R. M. *Curr. Opin. Chem. Biol.* **2008**, *12*, 491–496.
- (22) Wang, W.; Zhang, S. H.; Li, L. M.; Wang, Z. L.; Cheng, J. K.; Huang, W. H. *Anal. Bioanal. Chem.* **2009**, *394*, 17–32.
- (23) Amatore, C.; Arbault, S.; Guille, M.; Lemaitre, F. *Chem. Rev.* **2008**, *108*, 2585–2621.
- (24) Robinson, D. L.; Hermans, A.; Seipel, A. T.; Wightman, R. M. *Chem. Rev.* **2008**, *108*, 2554–2584.
- (25) Schroeder, T. J.; Jankowski, J. A.; Senyshyn, J.; Holz, R. W.; Wightman, R. M. *J. Biol. Chem.* **1994**, *269*, 17215–17220.
- (26) Robinson, I. M.; Finnegan, J. M.; Monck, J. R.; Wightman, R. M.; Fernandez, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 2474–2478.
- (27) Paras, C. D.; Qian, W. J.; Lakey, J. R.; Tan, W. H.; Kennedy, R. T. *Cell Biochem. Biophys.* **2000**, *33*, 227–240.
- (28) Wu, W. Z.; Huang, W. H.; Wang, W.; Wang, Z. L.; Cheng, J. K.; Xu, T.; Zhang, R. Y.; Chen, Y.; Liut, J. *J. Am. Chem. Soc.* **2005**, *127*, 8914–8915.
- (29) Li, Z. Y.; Zhou, W.; Wu, Z. X.; Zhang, R. Y.; Xu, T. *Biosens. Bioelectron.* **2009**, *24*, 1358–1364.
- (30) Cheng, H.; Huang, W. H.; Chen, R. S.; Wang, Z. L.; Cheng, J. K. *Electrophoresis* **2007**, *28*, 1579–1586.
- (31) Kim, K. T.; Koh, D. S.; Hille, B. *J. Neurosci.* **2000**, *20* (RC101), 1–5.
- (32) Billiard, J.; Koh, D. S.; Babcock, D. F.; Hille, B. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 12192–12197.
- (33) Mikeladze, E.; Schulte, A.; Mosbach, M.; Blochl, A.; Csoregi, E.; Solomon, R.; Schumann, W. *Electroanalysis* **2002**, *14*, 393–399.
- (34) Kurita, R.; Hayashi, K.; Horiuchi, T.; Niwa, O.; Maeyama, K.; Tanizawa, K. *Lab Chip* **2002**, *2*, 34–38.
- (35) Wang, J. *Analyst* **2005**, *130*, 421–426.
- (36) Pumera, M.; Sanchez, S.; Ichinose, I.; Tang, J. *Sens. Actuators, B: Chem.* **2007**, *123*, 1195–1205.

- (37) Kwon, K. Y.; Yang, S. B.; Kong, B. S.; Kim, J.; Jung, H. T. *Carbon* **2010**, *48*, 4504–4509.
- (38) Hou, S. F.; Kasner, M. L.; Su, S. J.; Patel, K.; Cuellar, R. *J. Phys. Chem. C* **2010**, *114*, 14915–14921.
- (39) Bratten, C. D. T.; Cobbold, P. H.; Cooper, J. M. *Anal. Chem.* **1998**, *70*, 1164–1170.
- (40) Chen, P.; Xu, B.; Tokranova, N.; Feng, X. J.; Castracane, J.; Gillis, K. D. *Anal. Chem.* **2003**, *75*, 518–524.
- (41) Gao, Y. F.; Chen, X. H.; Gupta, S.; Gillis, K. D.; Gangopadhyay, S. *Biomed. Microdevices* **2008**, *10*, 623–629.
- (42) Carabelli, V.; Gosso, S.; Marcantoni, A.; Xu, Y.; Colombo, E.; Gao, Z.; Vittone, E.; Kohn, E.; Pasquarelli, A.; Carbone, E. *Biosens. Bioelectron.* **2010**, *26*, 92–98.
- (43) Sun, X. H.; Gillis, K. D. *Anal. Chem.* **2006**, *78*, 2521–2525.
- (44) Amatore, C.; Arbault, S.; Chen, Y.; Crozatier, C.; Lemaitre, F.; Verchier, Y. *Angew. Chem., Int. Ed.* **2006**, *45*, 4000–4003.
- (45) Spegel, C.; Heiskanen, A.; Acklid, J.; Wolff, A.; Taboryski, R.; Emneus, J.; Ruzgas, T. *Electroanalysis* **2007**, *19*, 263–271.
- (46) Li, M. W.; Spence, D. M.; Martin, R. S. *Electroanalysis* **2005**, *17*, 1171–1180.
- (47) Dias, A. F.; Dernick, G.; Valero, V.; Yong, M. G.; James, C. D.; Craighead, H. G.; Lindau, M. *Nanotechnology* **2002**, *13*, 285–289.
- (48) Hafez, I.; Kisler, K.; Berberian, K.; Dernick, G.; Valero, V.; Yong, M. G.; Craighead, H. G.; Lindau, M. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 13879–13884.
- (49) Cui, H. F.; Ye, J. S.; Chen, Y.; Chong, S. C.; Sheu, F. S. *Anal. Chem.* **2006**, *78*, 6347–6355.
- (50) Isik, S.; Berdondini, L.; Oni, J.; Blochl, A.; Koudelka-Hep, M.; Schuhmann, W. *Biosens. Bioelectron.* **2005**, *20*, 1566–1572.
- (51) Cheng, W.; Klauke, N.; Sedgwick, H.; Smith, G. L.; Cooper, J. M. *Lab Chip* **2006**, *6*, 1424–1431.
- (52) Spegel, C.; Heiskanen, A.; Pedersen, S.; Emneus, J.; Ruzgas, T.; Taboryski, R. *Lab Chip* **2008**, *8*, 323–329.
- (53) Gao, Y. F.; Bhattacharya, S.; Chen, X. H.; Barizuddin, S.; Gangopadhyay, S.; Gillis, K. D. *Lab Chip* **2009**, *9*, 3442–3446.
- (54) Dittami, G. M.; Ayliffe, H. E.; King, C. S.; Rabbitt, R. D. *J. Microelectromech. Syst.* **2008**, *17*, 850–862.
- (55) Barizuddin, S.; Liu, X.; Mathai, J. C.; Hossain, M.; Gillis, K. D.; Gangopadhyay, S. *ACS Chem. Neurosci.* **2010**, *1*, 590–597.
- (56) Chen, X. H.; Gao, Y. F.; Hossain, M.; Gangopadhyay, S.; Gillis, K. D. *Lab Chip* **2008**, *8*, 161–169.
- (57) Dittami, G. M.; Rabbitt, R. D. *Lab Chip* **2010**, *10*, 30–35.
- (58) Gillis, K. D. In *Single Channel Recording*; Sakmann, B.; Neher, E., Eds.; Plenum Press: New York, 1995.
- (59) Chen, P.; Gillis, K. D. *Biophys. J.* **2000**, *79*, 2162–2170.
- (60) Fernandez, J. M.; Neher, E.; Gomperts, B. D. *Nature* **1984**, *312*, 453–455.
- (61) Bruggemann, A.; Stoelzle, S.; George, M.; Behrends, J. C.; Fertig, N. *Small* **2006**, *2*, 840–846.
- (62) Lau, A. Y.; Hung, P. J.; Wu, A. R.; Lee, L. P. *Lab Chip* **2006**, *6*, 1510–1515.
- (63) Dale, T. J.; Townsend, C.; Hollands, E. C.; Trezise, D. J. *Mol. BioSyst.* **2007**, *3*, 714–722.
- (64) Dunlop, J.; Bowlby, M.; Peri, R.; Vasilyev, D.; Arias, R. *Nat. Rev. Drug Discovery* **2008**, *7*, 358–368.
- (65) Farre, C.; George, M.; Brüggemann, A.; Fertig, N. *Drug Discovery Today: Technol.* **2008**, *5*, e23–e28.
- (66) Chen, P.; Hwang, T. C.; Gillis, K. D. *J. Gen. Physiol.* **2001**, *118*, 135–144.
- (67) Detoledo, G. A.; Fernandezchacon, R.; Fernandez, J. M. *Nature* **1993**, *363*, 554–558.
- (68) Chow, R. H.; Klingauf, J.; Heinemann, C.; Zucker, R. S.; Neher, E. *Neuron* **1996**, *16*, 369–376.
- (69) Albillas, A.; Dernick, G.; Horstmann, H.; Almers, W.; de Toledo, G. A.; Lindau, M. *Nature* **1997**, *389*, 509–512.
- (70) Dernick, G.; Gong, L. W.; Tabares, L.; de Toledo, G. A.; Lindau, M. *Nat. Methods* **2005**, *2*, 699–708.
- (71) Huang, Y. X.; Chen, P. *Adv. Mater.* **2010**, *22*, 2818–2823.
- (72) Noy, A.; Artyukhin, A. B.; Misra, N. *Mater. Today* **2009**, *12*, 22–31.
- (73) Pui, T. S.; Sudibya, H. G.; Luan, X. N.; Zhang, Q.; Ye, F.; Huang, Y. X.; Chen, P. *Adv. Mater.* **2010**, *22*, 3199–3203.
- (74) Patolsky, F.; Timko, B. P.; Yu, G. H.; Fang, Y.; Greytak, A. B.; Zheng, G. F.; Lieber, C. M. *Science* **2006**, *313*, 1100–1104.
- (75) Wang, C. W.; Pan, C. Y.; Wu, H. C.; Shih, P. Y.; Tsai, C. C.; Liao, K. T.; Lu, L. L.; Hsieh, W. H.; Chen, C. D.; Chen, Y. T. *Small* **2007**, *3*, 1350–1355.
- (76) Tsai, C. C.; Yang, C. C.; Shih, P. Y.; Wu, C. S.; Chen, C. D.; Pan, C. Y.; Chen, Y. T. *J. Phys. Chem. B* **2008**, *112*, 9165–9173.
- (77) Huang, Y. X.; Sudibya, H. G.; Fu, D. L.; Xue, R. H.; Dong, X. C.; Li, L. J.; Chen, P. *Biosens. Bioelectron.* **2009**, *24*, 2716–2720.
- (78) Sudibya, H. G.; Ma, J. M.; Dong, X. C.; Ng, S.; Li, L. J.; Liu, X. W.; Chen, P. *Angew. Chem., Int. Ed.* **2009**, *48*, 2723–2726.
- (79) Pui, T. S.; Agarwal, A.; Ye, F.; Balasubramanian, N.; Chen, P. *Small* **2009**, *5*, 208–212.
- (80) Pui, T. S.; Agarwal, A.; Ye, F.; Ton, Z. Q.; Huang, Y. X.; Chen, P. *Nanoscale* **2009**, *1*, 159–163.
- (81) Pui, T. S.; Agarwal, A.; Ye, F.; Huang, Y. X.; Chen, P. *Biosens. Bioelectron.* **2011**, *26*, 2746–2750.
- (82) Gong, J. R. *Small* **2010**, *6*, 967–973.
- (83) Choi, A.; Kim, K.; Jung, H. I.; Lee, S. Y. *Sens. Actuators, B: Chem.* **2010**, *148*, 577–582.
- (84) Ishikawa, F. N.; Chang, H. K.; Curreli, M.; Liao, H. I.; Olson, C. A.; Chen, P. C.; Zhang, R.; Roberts, R. W.; Sun, R.; Cote, R. J.; Thompson, M. E.; Zhou, C. W. *ACS Nano* **2009**, *3*, 1219–1224.
- (85) Huang, Y. X.; Dong, X. C.; Shi, Y. M.; Li, C. M.; Li, L. J.; Chen, P. *Nanoscale* **2010**, *2*, 1485–1488.
- (86) Dong, X. C.; Shi, Y. M.; Huang, W.; Chen, P.; Li, L. J. *Adv. Mater.* **2010**, *22*, 1649–1653.
- (87) Yang, W. R.; Ratnac, K. R.; Ringer, S. P.; Thordarson, P.; Gooding, J. J.; Braet, F. *Angew. Chem., Int. Ed.* **2010**, *49*, 2114–2138.
- (88) He, Q. Y.; Sudibya, H. G.; Yin, Z. Y.; Wu, S. X.; Li, H.; Boey, F.; Huang, W.; Chen, P.; Zhang, H. *ACS Nano* **2010**, *4*, 3201–3208.
- (89) Stern, E.; Vacic, A.; Li, C.; Ishikawa, F. N.; Zhou, C. W.; Reed, M. A.; Fahmy, T. M. *Small* **2010**, *6*, 232–238.
- (90) Xue, R. H.; Zhao, Y. Y.; Su, L. Y.; Ye, F.; Chen, P. *Pflug. Arch. Eur. J. Phys.* **2009**, *458*, 1137–1149.
- (91) Holz, R. W.; Axelrod, D. *Acta Physiologica* **2008**, *192*, 303–307.
- (92) Zhang, E. M.; Xue, R. H.; Soo, J.; Chen, P. *Pflügers Archiv European Journal of Physiology* **2008**, *457*, 211–222.
- (93) Ye, F.; Than, A.; Zhao, Y. Y.; Goh, K. H.; Chen, P. *J. Endocrinol.* **2010**, *206*, 27–36.
- (94) Zhang, J.; Xue, R. H.; Ong, W. Y.; Chen, P. *Biophys. J.* **2009**, *97*, 1371–1380.
- (95) Than, A.; Ye, F.; Xue, R. H.; Ong, J. W.; Poh, C. L.; Chen, P. *Mol. Cell. Endocrinol.* **2011**, *332*, 261–270.
- (96) Degtyar, V. E.; Allersma, M. W.; Axelrod, D.; Holz, R. W. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 15929–15934.
- (97) Huang, B.; Babcock, H.; Zhuang, X. W. *Cell* **2010**, *143*, 1047–1058.
- (98) Willig, K. I.; Rizzoli, S. O.; Westphal, V.; Jahn, R.; Hell, S. W. *Nature* **2006**, *440*, 935–939.
- (99) Westphal, V.; Rizzoli, S. O.; Lauterbach, M. A.; Kamin, D.; Jahn, R.; Hell, S. W. *Science* **2008**, *320*, 246–249.
- (100) Sieber, J. J.; Willig, K. I.; Kutzner, C.; Gerding-Reimers, C.; Harke, B.; Donnert, G.; Rammner, B.; Eggeling, C.; Hell, S. W.; Grubmuller, H.; Lang, T. *Science* **2007**, *317*, 1072–1076.
- (101) Michalet, X.; Pinaud, F. F.; Bentolila, L. A.; Tsay, J. M.; Doose, S.; Li, J. J.; Sundaresan, G.; Wu, A. M.; Gambhir, S. S.; Weiss, S. *Science* **2005**, *307*, 538–544.
- (102) Liu, A. P.; Peng, S.; Soo, J. C.; Kuang, M.; Chen, P.; Duan, H. W. *Anal. Chem.* **2011**, *83*, 1124–1130.
- (103) Zhang, Q.; Li, Y. L.; Tsien, R. W. *Science* **2009**, *323*, 1448–1453.
- (104) Nechyboruk-Zloy, V.; Dieterich, P.; Oberleithner, H.; Stock, C.; Schwab, A. *Am. J. Physiol. Cell Physiol.* **2008**, *294*, C1096–C1102.
- (105) Pinaud, F.; Michalet, X.; Iyer, G.; Margeat, E.; Moore, H. P.; Weiss, S. *Traffic* **2009**, *10*, 691–712.

- (106) Saha, A.; Basiruddin, S. K.; Sarkar, R.; Pradhan, N.; Jana, N. R. *J. Phys. Chem. C* **2009**, *113*, 18492–18498.
- (107) Beaurepaire, E.; Buissette, V.; Sauviat, M. P.; Giaume, D.; Lahil, K.; Mercuri, A.; Casanova, D.; Huignard, A.; Martin, J. L.; Gacoin, T.; Boilot, J. P.; Alexandrou, A. *Nano Lett.* **2004**, *4*, 2079–2083.
- (108) Blechinger, J.; Herrmann, R.; Kiener, D.; Garcia-Garcia, F. J.; Scheu, C.; Reller, A.; Brauchle, C. *Small* **2010**, *6*, 2427–2435.
- (109) Jena, B. P. *Ultramicroscopy* **2006**, *106*, 663–669.
- (110) Shin, W.; Gillis, K. D. *Biophys. J.* **2006**, *91*, L63–L65.
- (111) Wittstock, G.; Burchardt, M.; Pust, S. E.; Shen, Y.; Zhao, C. *Angew. Chem., Int. Ed.* **2007**, *46*, 1584–1617.
- (112) Bauermann, L. P.; Schuhmann, W.; Schulte, A. *Phys. Chem. Chem. Phys.* **2004**, *6*, 4003–4008.
- (113) Isik, S.; Schuhmann, W. *Angew. Chem., Int. Ed.* **2006**, *45*, 7451–7454.
- (114) Takahashi, Y.; Shiku, H.; Murata, T.; Yasukawa, T.; Matsue, T. *Anal. Chem.* **2009**, *81*, 9674–9681.
- (115) Stybayeva, G.; Kairova, M.; Ramanculov, E.; Simonian, A. L.; Revzin, A. *Colloids Surf, B* **2010**, *80*, 251–255.
- (116) Chou, T. H.; Chuang, C. Y.; Wu, C. M. *Cytokine* **2010**, *51*, 107–111.
- (117) Andersson, H.; Kagedal, B.; Mandenius, C. F. *Anal. Bioanal. Chem.* **2010**, *398*, 1395–1402.
- (118) Endo, T.; Yamamura, S.; Kerman, K.; Tamiya, E. *Anal. Chim. Acta* **2008**, *614*, 182–189.
- (119) Mazzotta, F.; Wang, G. L.; Haglund, C.; Hook, F.; Jonsson, M. P. *Biosens. Bioelectron.* **2010**, *26*, 1131–1136.
- (120) Banerjee, P.; Bhunia, A. K. *Trends Biotechnol.* **2009**, *27*, 179–188.
- (121) Ding, L.; Du, D.; Zhang, X. J.; Ju, H. X. *Curr. Med. Chem.* **2008**, *15*, 3160–3170.
- (122) Michelini, E.; Cevenini, L.; Mezzanotte, L.; Coppa, A.; Roda, A. *Anal. Bioanal. Chem.* **2010**, *398*, 227–238.
- (123) Kintzios, S. E. *Mini-Rev. Med. Chem.* **2007**, *7*, 1019–1026.
- (124) Nakamura, M.; Mie, M.; Funabashi, H.; Yamamoto, K.; Ando, J.; Kobatake, E. *Anal. Biochem.* **2006**, *352*, 61–67.
- (125) Pangrsic, T.; Potokar, M.; Stenovec, M.; Kreft, M.; Fabbretti, E.; Nistri, A.; Pryazhnikov, E.; Khiroug, L.; Giniatullin, R.; Zorec, R. *J. Biol. Chem.* **2007**, *282*, 28749–28758.
- (126) Okumoto, S.; Looger, L. L.; Micheva, K. D.; Reimer, R. J.; Smith, S. J.; Frommer, W. B. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 8740–8745.
- (127) Nguyen, Q. T.; Schroeder, L. F.; Mank, M.; Muller, A.; Taylor, P.; Griesbeck, O.; Kleinfeld, D. *Nat. Neurosci.* **2010**, *13*, 127–132.
- (128) Soo, J. C.; Zhang, J.; He, Q. Y.; Agarwal, S.; Li, H.; Zhang, H.; Chen, P. *Integr. Biol.* **2010**, *2*, 250–257.
- (129) Zheng, X. T.; Yang, H. B.; Li, C. M. *Anal. Chem.* **2010**, *82*, 5082–5087.
- (130) Sun, P.; Laforge, F. O.; Abeyweera, T. P.; Rotenberg, S. A.; Carpino, J.; Mirkin, M. V. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 443–448.
- (131) Singhal, R.; Orynbayeva, Z.; Sundaram, R. V. K.; Niu, J. J.; Bhattacharyya, S.; Vitol, E. A.; Schrlau, M. G.; Papazoglou, E. S.; Friedman, G.; Gogotsi, Y. *Nat. Nanotechnol.* **2011**, *6*, 57–64.
- (132) Tian, B. Z.; Cohen-Karni, T.; Qing, Q. A.; Duan, X. J.; Xie, P.; Lieber, C. M. *Science* **2010**, *329*, 830–834.
- (133) Brashear, A. *Semin. Neurol.* **2001**, *21*, 85–90.