TOXICOLOGICAL SCIENCES **74,** 235–244 (2003)
DOI: 10.1093/toxsci/kfg108

REVIEW

Shrinking the Biologic World—Nanobiotechnologies for Toxicology

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Received January 2, 2003; accepted March 29, 2003

Although toxicologic effects need to be considered at the organismal level, the adverse events originate from interactions and alterations at the molecular level. Cellular structures and functions can be disrupted by modifications of the nanometer structure of critical molecules; therefore, devices used to assess biologic and toxicologic processes at the nanoscale will allow important new research pursuits. In order to properly assess alterations at these dimensions, nanofabricated tools are needed to detect, separate, analyze, and manipulate cells or biologic molecules of interest. The emergence of laser tweezers, surface plasmon resonance (SPR), laser capture microdissection (LCM), atomic force microscopy (AFM), and multi-photon microscopes have allowed for these assessments. Micro- and nanobiotechnologies will further advance biologic, clinical, and toxicologic endeavors with the aid of miniaturized, more sensitive devices. Miniaturized table-top laboratory equipment incorporating additional innovative technologies can lead to new advances, including micro total analysis systems (μTAS) or "lab-on-a-chip" and "sentinel sensor" devices. This review will highlight several devices, which have been made possible by techniques originating in the microelectronics industry. These devices can be used for toxicologic assessment of cellular structures and functions, such as cellular adhesion, signal transduction, motility, deformability, metabolism, and secretion.

Key Words: **nanofabricated tools; laser tweezers; surface plasmon resonance; laser capture microdissection; atomic force microscopy; multi-photon microscopes.**

Smaller, faster, cheaper, and more sensitive: these are the hallmarks of new research tools that have applications in biologic, clinical, and toxicologic research. Combinatorial chemistry, microarrays, high-throughput screening assays, and portable biosensors are all examples of newly available technologies, but they only foreshadow the tools and techniques on the horizon and the impact that these tools will have for our understanding of biologic systems. While miniaturization has intrinsic advantages for reducing reagent use, increasing sensitivity, improving resolution time, and allowing for higher sample throughput, miniaturization can also reveal entirely new aspects of biologic systems. The trend to shrink instrumentation enables the study of biologic phenomena at the nanometer scale and will dramatically change our view of fundamental biologic systems and the impact of toxicants on those systems. Newly devised nanobiotechnologies will ultimately serve to connect our understanding of biologic systems at the macroscopic level to the biochemical characterization of these same systems at the nano level.

At their root, biologic mechanisms all operate at a molecular (nanoscale) level. Thus, cellular differentiation, communication, motility, and other complex events that are usually measured at some macro-scale level are in fact regulated by individual molecular events that operate in the nanoscale. Investigations that consider biologic events on the scale of individual molecules are collectively termed nanobiotechnology, though this term is used with considerable flexibility in the literature. For our purposes, nanobiotechnology will be used to describe assay systems and manipulations of the physical world at the nanometer and micrometer scale with the intent of detecting, separating, analyzing, manipulating, and characterizing cells or biologic molecules of interest. This review will highlight several micro- and nano-fabricated devices and systems and their use in the assessment of toxicant effects on individual and coordinated cellular structures and functions, such as cellular adhesion, signal transduction, motility, deformability, metabolism, and secretion.

The production of these micro- and nano-scale devices often depends on a range of fabrication techniques originally devised for the microelectronics industry, including processes such as mask production with computer aided design (CAD), etching, thin-film deposition, photolithography, and soft lithography (Chen and Pepin, 2001; Whitesides *et al.,* 2001). Devices are designed and fabricated using a variety of biocompatible materials, such as silicon, glass, polydimethylsiloxane, and polystyrene that contain the desired topographical features. Once constructed, these devices can be further modified with bio-

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logic molecules, which can render a particular surface biologically active or inactive, with the direct application of molecules to the substrate by bath application, microfluidic networks (Delamarche *et al.,* 1997), laminar flow deposition (Takayama *et al.,* 1999, 2001), micro-contact printing (Bernard *et al.,* 1998; Degenaar *et al.,* 2001; Kane *et al.,* 1999), self-assembling monolayers (Tan *et al.,* 2002), or a combination of the above (St. John *et al.,* 1997). Patterning of biologic molecules, for example, can promote direct and selective cellular attachment to a substrate in a complimentary area or pattern (Kam *et al.,* 2002; St. John *et al.,* 1997) as well as direct outgrowth from cell bodies, such as neuronal axons and dendrites (Esch *et al.,* 1999; James *et al.,* 2000). Extracellular matrix (ECM) proteins can be patterned to guide cellular chemotaxis or haptotaxis, which is movement in response to a soluble or structural gradient, respectively (Perumpanani *et al.,* 1998).

Consider, for example, the trafficking of blood leukocytes (lymphocytes, monocytes, and neutrophils) to infectious foci. Chemoattractants generated in the inflamed tissue diffuse away from the inflammation and establish a shallow three-dimensional gradient that orients (polarizes) the responding cells. These chemoattractants, which bind to the luminal side of the endothelial cells, activate conformational changes in adhesion molecules such as ICAM-1 in order to increase leukocyte: endothelial adhesion and to offset the shear forces of the blood flow, allowing eventual extravasation (diapedesis) of the leukocytes from the lumen of the blood vessel into the inflamed tissue. During diapedesis and once in the tissue matrix, the responding cells must deform to move through the ECM as they make their way toward higher concentrations of chemoattractant. Micro- and nano-devices have been designed and fabricated to model and quantify each step of this physiologic process.

Normal human leukocytes range in size from 6–15 μm in diameter. Therefore, even during normal blood flow through the microvasculature, the cells must be able to deform to pass through capillaries that can be significantly smaller than the cell. A widely used method to quantify rigidity of cells is the micropipet aspiration technique (Melder and Jain, 1994; Sasaki *et al.,* 1989). This method utilizes negative pressure to pull a portion of the cell membrane into a micropipet that has an internal diameter in the range of 2–6 μm. Using a mathematical model, viscoelastic coefficients for the membrane and cytoplasm can be calculated (Sasaki *et al.,* 1989). This technique offers valuable information on localized rigidity of the cell membrane and associated cytoplasmic structures; however, information about a cell's viscoelastic properties does not directly relate to the cell's ability to deform as a whole in order to flow through capillaries, nor to extravasate. Such processes are thought to require energy, cytoskeletal reorganization, and changes in membrane fluidity (Worthylake *et al.,* 2001). The manufacture of structures that model those found *in vivo* (biomimicry) allow us to evaluate how a cell deforms as a whole under a variety of normal and abnormal conditions. Channels of varying size can be constructed to represent the diameter of a capillary ($\sim$ 4 to 9 μm), through which individual cells must pass under physiologic fluid flow conditions (Carlson *et al.,* 1998). These types of devices have been used to show that cellular parameters such as cell type and nuclear morphology can have significant effects on cellular trafficking through capillaries (Carlson *et al.,* 1998). This device could also give important information about the cellular trafficking capabilities of leukocytes under a variety of other conditions, such as how leukocytes from aged individuals differ from young individuals, how leukocytes exposed to oxidative stress differ from normal leukocytes or those exposed to antioxidants or reducing agents. These important properties could influence leukocyte trafficking, ultimately having profound effects on an individual's ability to mount a rapid and effective immune response to infection.

Another critical element in cellular movement is the attachment of cells to the substrate across and through which they must move. Cell adhesion to surfaces has been studied by measurements of the forces necessary to displace populations of cells from a surface (Pelham and Wang, 1998). Cellular adhesion has also been studied using Electric Cell Impedance Sensor (ECIS) technology, which is comprised of a photolithographed electrode pair on a surface with which cells interact (Giaever and Keese, 1984; Kowolenko *et al.,* 1990). Cells placed on the small (3×10^{-4} cm^2) sensing electrode impede a small (1 volt) a.c. current that is passed through these electrodes. As the cells respond to external stimuli, the intimacy of contact with the electrode surface changes and the impedance changes accordingly. Macrophages activated by lipopolysaccharide will spread to cover more of the sensing electrode surface, increasing resistance dramatically (Kowolenko *et al.,* 1990). Conversely, as cells are exposed to toxicants, they may undergo morphological changes, apoptosis, or necrotic cell death, detaching from the surface, rounding up, and losing their ability to impede current flow, as would be seen with macrophages exposed to *B. anthracis* (Park *et al.,* 2002).

The ECIS system has more recently been modified to assess directional cell movement (ECIS/taxis; Hadjout *et al.,* 2001). In this configuration, the electrodes are placed in the path of cells responding to a chemoattractant gradient. As the cells crawl in a chemotactic gradient stabilized by a gel matrix, they traverse the target electrode and cause an increase in resistance to current flow (Fig. 1). This change in the electrical behavior of the circuit can be used to calculate speed of cell movement in response to the chemoattractant. The impact of pharmaceuticals and toxicants on the chemotactic response are readily measured by this technology. The system is sensitive enough to detect the arrival of a single cell at the target electrode, making assessments of very small populations of cells possible. Further adaptations of the ECIS/taxis system, such as the use of multiple small linear detection electrodes in parallel instead of a

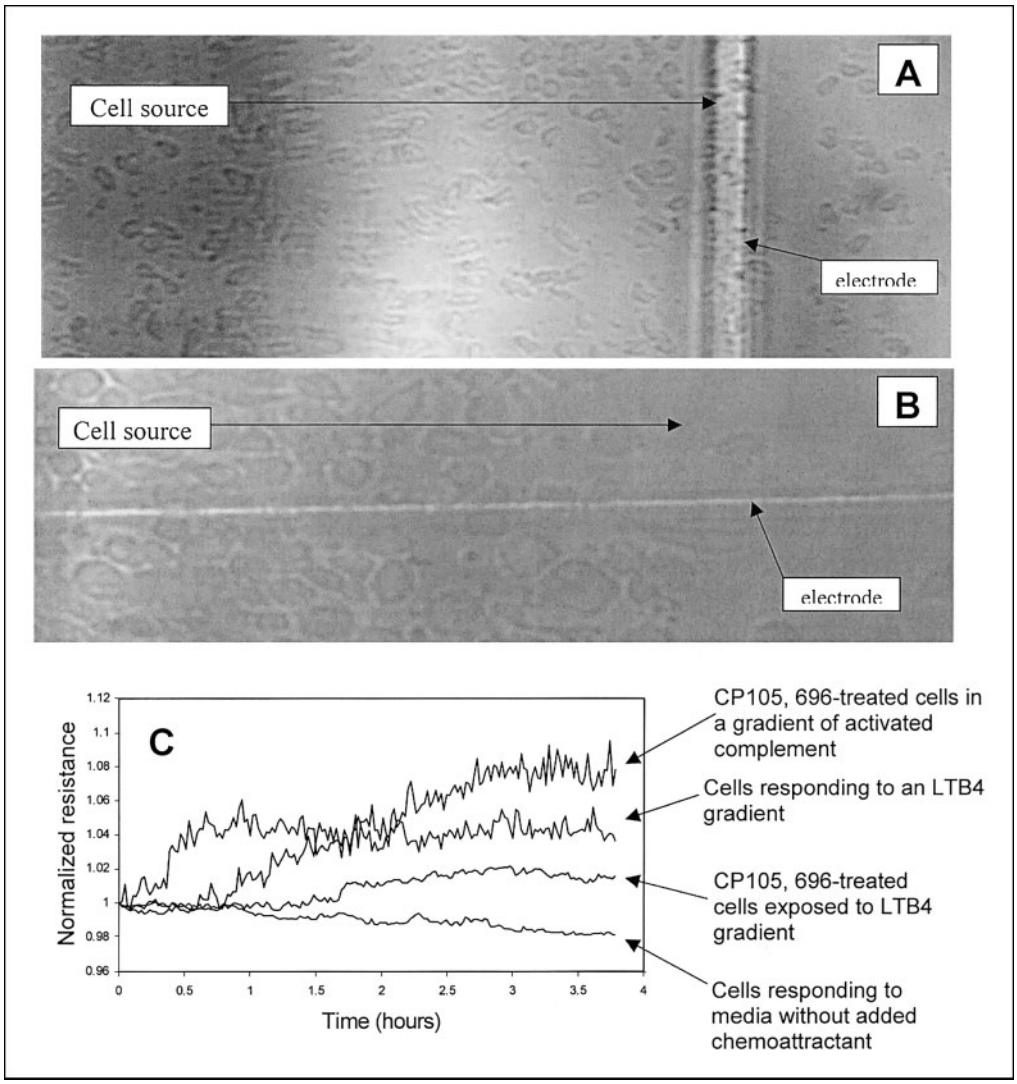

FIG. 1. ECIS/taxis measurements of cell movement. (A) Cells responding to a chemotactic gradient move in the direction of the arrow and ultimately cross the micro-electrode that is oriented perpendicular to the axis of cell movement. Crossing the electrode causes a change in current flow through the electrode that is recorded as an increased resistance. Higher resistance values correlate with greater coverage of the electrode by cells. (B) Cells responding to a chemotactic gradient move in the direction of the arrow. In this configuration, the cells move along the long axis of the electrode, and cover increasing amounts of the electrode as they move further from the cell origin. (C) Normalized resistance (Y-axis) is plotted against time (X-axis). Cells cultured in the absence of chemoattractant do not move to the electrode and thus do not alter the electrode resistance. Cells cultured in a gradient of activated complement, or in a gradient of leukotriene B4 (LTB4) display a vigorous response, and arrive at the target electrode. Cells pretreated with an experimental LTB4 antagonist (CP105,696; generously provided by Pfizer, Inc.) display a dramatically diminished and slowed response to chemoattractant.

single circular target electrode, can yield information on the chemotactic responses of individual cells within the responding population, such as persistence of directional movement and the response to complex and overlapping gradients of chemoattractant agonists and antagonists.

Other electrode configurations have also been used with success. For example, an electrode sized to approximate the same dimensions as an individual cell has been used to monitor neuronal cell function (Pancrazio *et al.,* 1998). In this case, extracellular biopotentials can be recorded from individual cells under normal culture conditions and when exposed to neuronal toxicants, such as ion channel antagonists or blockers (i.e., verapamil and tetrodotoxin; Pancrazio *et al.,* 1998). This technology enables the assessment of individual cell behavior instead of the assessment of population behavior.

Cellular adhesion, diapedesis, and chemotaxis are all coordinated events during an immune response; therefore, it would be interesting to evaluate all of these parameters simultaneously. Barrier walls with nano- and microscale gaps, simulating the fenestra between vascular endothelial cells, can be fabricated in polystyrene to model these mechanical gaps through which cells must pass. *In vivo* fenestration sizes vary depending on the organ or tissue and can range from 20–3000 nm (Ganong, 1993). Figure 2 displays a barrier with 5 μm portals fabricated in polystyrene to assess cell deformability during chemokinesis. This type of device allows for the direct observation and imaging of individual and coordinated cellular movements during chemotaxis in real time, using time-lapse microscopy. These barrier walls when used in combination with the under-agarose chemotaxis assay (Nelson *et al.,* 1975), or the ECIS/taxis assay can be used to evaluate a cell's ability to chemotax and to deform in a three-dimensional *in vitro* model of cell movement.

A significant nano-scale component of cellular adhesion, diapedesis and chemotaxis is the formation and breaking of focal contacts. An increase in adhesive strength at the leading

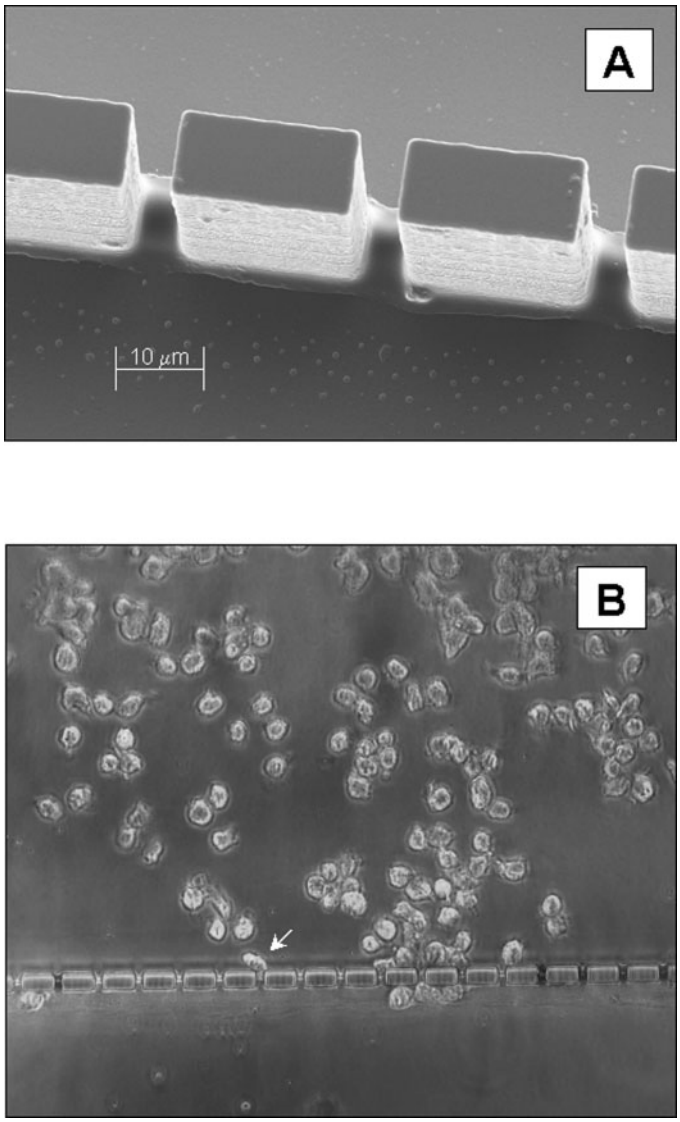

FIG. 2. Microfabricated system to simulate cell deformability as occurs during diapedesis in response to a chemokine gradient. SEM of a polystyrene chemotaxis/chemokinesis chamber barrier wall ("picket fence") is shown, which is 20 μm high with 5 μm channels bisecting a 9 $\times$ 9 mm chamber (A). In this system, the movement of a human peripheral leukocyte/mouse macrophage hybridoma cell line (WBC264-9C) can be assayed in response to C5a being generated on the opposite side of the barrier (B). For movies of the cell movement (Figs. 1 and 2), go to the supplementary data for this article on the journal's Web site.

edge is the result of an increase in the number of focal adhesions, an increase in the strength of individual focal adhesions (via dephosphorylation of the B1 subunit of the integrin), or a combination of the two. Trans-membrane proteins, called integrins, have been shown to localize at the leading edge and their extracellular domains bind to the ECM. Intracellularly, stress fibers made up of F-actin are formed at the site of the focal adhesion and are attached to the cytosolic domain of the integrin that spans the extracellular and intracellular compartments. Using a recently developed polyacrylamide substratum (Pelham and Wang, 1998), force generation at the focal adhesion can be measured using a novel method called traction force microscopy, a method of computational procedures that convert measurements of substrate deformation of a resilient surface into an estimate of traction stresses. These substrate deformations can then be viewed as time-lapse images. As shown for fibroblast migration, shear fields of traction stress at high temporal and spatial resolution can be determined (Munevar *et al.,* 2001). Toxins that affect force application through focal adhesions can readily be assessed by the subtle changes they may evoke in these nanoscale measurements of force.

The chemotactic gradients that are responsible for directional movement of leukocytes are transient and ill defined in traditional macro-scale measures of chemotaxis. Thus, it is difficult to assess the actual conditions to which a cell is exposed. A novel approach allows the precise definition of the conditions under which the cell responds to chemoattractants. Microlithographic techniques allow for the construction of Y-shaped flow chambers with a Reynolds number (a measure of turbulence) that is low enough to preclude mixing of reagents that flow through the chamber (Takayama *et al.,* 1999, 2001). Chemoattractant agents introduced into the separate arms of the chamber will remain distinct laminar flows through the chamber. Individual cells placed at the interface of these agents can be observed for specific activities as the composition of the adjacent flows is manipulated. Laminar flow also allows for the generation of complex chemokine gradients that can be spatially and temporally controlled. Differential fluidics allow establishment of a "hill" gradient (0–100%—100–0%) versus a "cliff" gradient (0–100%—0–100%), which differentially affects cell migration (Jeon *et al.,* 2002). Similarly, agonists or antagonists can be inserted into the gradients at precise locations. In each of these single cell assessments, the role of toxin and toxicant can be explored in as yet untapped ways.

Biologists are also taking advantage of optical properties to study nanoscale interactions. Optical tweezers, used to manipulate cells and particles, surface plasmon resonance (SPR), used to measure the interaction between ligands and receptors, and laser capture microdissection (LCM), used to select particular cells from a tissue dissection are each contributing to the shrinking scale of biological assessments. Optical tweezers are three-dimensional traps that use a focused laser (with a wavelength range of 700–1000 nm and a power of 25 to 500 mW) to manipulate microscopic objects. These wavelengths are refracted, but not absorbed, by biological materials. The vertical light, with zero horizontal momentum, is refracted when passed through a sphere (such as a cell) and a horizontal momentum gradient is established within the sphere due to the asymmetry of the light (Brown, 2001). This creates an imbalance in the reaction forces and the object is pulled toward the brighter side of the light. The effect of the laser on the object

is dependent upon the wavelength and power of the laser as well as the shape (spheroid vs. nonspheroid) and size (tens of nanometers to many micrometers) of the object (Block, 1992). Either moving the laser or the stage can manipulate the trapped object. Laser tweezers can be used to study surface dynamics and centripetal movement of engulfed particles, for example, by manipulating beads, fibronectin-, or concavalin A conjugated beads, which bind to cellular integrins or carbohydrate moieties, respectively, on the cell surface (Caspi *et al.,* 2001). When the beads are placed near a cell surface using the optical tweezers, the cell pulls beads of either type out of the optical trap in less than 1 min or immediately after adhesion if the optical trap is extinguished. Optical tweezers can also be used to apply a force on a cell membrane in order to measure changes in cellular function. Exposure of polymorphonuclear lymphocytes to an optical tweezer resulted in the opening of a mechanically inducible, membrane channel-mediated influx of extracellular calcium (Holm *et al.,* 1999).

SPR provides a label-free, real-time measurement of specific protein interactions, e.g., antibody-antigen interaction using an optical reflectance method that detects changes in the optical properties of an aqueous medium close to a gold surface. These properties change as the mass at the gold/medium interface changes. The mass at an interface can increase due to the specific capture of an antigen by its partner antibody or nonspecific adsorption of proteins or blocking agents to the gold surface. A surface plasmon itself is an electromagnetic field charge-density oscillation that exists at a metal-dielectric interface (Geddes *et al.,* 1994). One common technique used to achieve resonance is the Kretschmann configuration, which uses a prism to match the phase velocities of the incident light and the surface plasmon. In this configuration, a glass slide is covered with a thin layer of vapor-deposited gold and is brought into optical contact with the prism using a thin layer of refractive index matching fluid (Geddes *et al.,* 1994). A resonant minimum is observed when the incident light is at a specific angle to the gold surface because energy from the incident light is coupled into the surface plasmon. This resonant angle increases as the mass at the interface increases. SPR is currently being used to measure antibody-antigen interactions (Rasooly, 2001; Roggenbuck *et al.,* 1994; Visser and Smit-Kingma, 1999) and binding kinetics (Rich and Myszka, 2001) as well as to differentiate cell types using antibodies to particular cell surface markers (Quinn *et al.,* 1997).

A microscale, multiplexed version of SPR is grating-coupled SPR (GC-SPR). GC-SPR achieves the essential matching of phase velocities using an optical grating embossed on the chip surface as a substitute for the prism. When the chip is exposed to p-polarized, incident light at a particular wavelength and angle, energy from the incident light resonantly couples with the electrons of the metal, causing them to oscillate together as a single entity and generate a surface plasmon (Brockman and Fernandez, 2001). This resonance condition results in a drop, or minima, in the reflectance of the incident light and its angle is considered the SPR angle. A 1 cm^2 GC-SPR chip array can contain thousands of individual regions of receptors, which can be simultaneously monitored with an imaging detector, such as a charge-coupled device (CCD) camera (Brockman and Fernandez, 2001). These chips can be used as protein microarrays to simultaneously detect the presence of hundreds of proteins within cell lysate or serum. This technology offers significant advantages in the simultaneous inspection of hundreds of proteins that may reflect aspects of the overall biological response to toxicants.

LCM utilizes an infrared laser integrated with a standard microscope. A thermoplastic transparent membrane is placed on top of a prepared tissue section on a glass slide. When the cells of interest are in the field of vision, the laser is turned on and the thermoplastic membrane is activated, resulting in the subsequent binding and procurement of the cells of interest. LCM is currently being used to capture and study single B cells after gene rearrangement (Obiakor *et al.,* 2002), the role of interferon gamma in the recognition of melanoma cells by T lymphocytes (Obiakor *et al.,* 2002), and the potential to capture and separate normal prostate and cancer cells according to their cluster of differentiation (CD) markers (Liu and True, 2002). Again, subtle cellular features altered by the presence of toxicant (but obscured in a background of unaffected cells) may become evident when LCM harvests the individual cells.

Atomic force microscopy (AFM) is a nanotechnology that can be used to study the structure and properties of cellular membranes (Dufrene, 2001; Kaasgaard *et al.,* 2002), the cytoskeletal network (Nowakowski *et al.,* 2001), and ligand-receptor interaction (Da Silva, 2002; Dufrene, 2001), at the nanoscale level. AFM works by measuring a local property, such as height, with a probe placed very close to the sample. As the probe is displaced by changes in the sample topography, laser light refraction by the probe is altered proportionately. AFM can achieve a vertical resolution of picometers and a horizontal resolution of nanometers and can image samples in air and in aqueous solutions that simulate physiological conditions. This rivals the resolution of the cryogenic transmission electron microscope with the advantage of scanning living cells (Allen *et al.,* 1993, 1995).

Traditional AFM methods are currently being improved through enhancements in the tip structure used for probing the cellular surface, since tip size ultimately determines the resolution of the image. The most commonly used tips in AFM consist of poorly characterized silicon and silicon nitride. New tips consisting of carbon nanotubes are currently being developed. These consist of a honeycomb sp^2 hybridized carbon network that is rolled into a seamless cylinder (Hafner *et al.,* 2001). The carbon nanotubes can consist of single-walled or multi-walled nanotubes that range from 0.35 to 50 nm. The nanotubes have an extremely small diameter, a high aspect ratio, a high Young's modulus, and elastically buckle under heavy loads (Hafner *et al.,* 2001) making them extremely

suitable for probing the surface of a cell, and for mapping ultrastructural changes evoked by toxins.

Another important nanoscale parameter of toxicologic assessment is modulation of exocytosis by various cell types. Upon stimulation, a variety of molecules, such as neurotransmitters, hormones, peptides, and cytokines, are released from cells into synaptic clefts, intercellular spaces, and/or the circulation. Numerous environmental agents can interfere with exocytotic mechanisms. For example, tetanus and botulinum toxins exert their toxic effects by preventing the docking of vesicles to the plasma membrane, thereby inhibiting exocytosis (Humeau *et al.,* 2000). A microfabricated electrochemical detector array has been developed to study the mechanisms of exocytosis (Dias *et al.,* 2002). This device consists of an amperometric four-detector array on a glass cover slip, which contains a $10 \times 10\ \mu m^2$ well created in photoresist. The photoresist serves a dual function as an insulator and as the structure of the well that contains the individual cell (Dias *et al.,* 2002). This device is sensitive to a single vesicular release event and allows for spatial and temporal resolution of releasing events at the level of the individual cell (Dias *et al.,* 2002). This device has the potential to rapidly screen numerous agonists, antagonists, and potential toxicants and their effect on exocytosis from a variety of cell types simultaneously and in real time. When electrochemical and optical analysis are interfaced, the overall evaluations will be even more powerful.

Nanobiotechnology also enables us to envision the physical aspects of macromolecular processes. For example, determining the weight of a single cell or bacteria is now possible, which could allow detection of mass changes resulting from stimulatory or toxic events. In contrast to weight loss, weight gain as would occur with the phagocytosis of pathogens by a single cell could be quantified; possibly, even modulation of ligand binding could be assayed. Such weight measurements can only be done using nanoscale balances sensitive to at least the femtogram level. Microelectromechanical (MEMs) and nanoelectromechanical systems (NEMs) are being developed to enable these measurements. MEMs and NEMs are mechanical devices that transduce changes in mechanical properties into optical or electrical signals. NEMs oscillators are resonant frequency-based detection sensors that sense changes in an established resonant frequency based on the mass applied. These sensors can be used as a mass-based biosensor by applying capture antibodies able to bind a specific organism to the oscillator (Ilic *et al.,* 2000). Upon pathogen binding, the mechanical device will register the increase in mass. These nanomechanical resonant frequency oscillators are highly sensitive and already have been shown to be sensitive enough to detect the presence of an antibody monolayer and a single *Escherichia coli* (*E. coli*) cell calculated to weigh 665 femtograms (Ilic *et al.,* 2001). Future biologic processes may be measurable by mass analysis.

Biosensor devices are also shrinking in scale. These devices must provide three integrated processes: a recognition step, which detects biologic and chemical analytes in solution or in the atmosphere; a transduction step, which produces and amplifies the signal to be detected; and a detection device, which can translate the signal into qualitative or quantitative information. Biosensors have been categorized as molecule-, whole cell-, and whole organ/tissue-devices, based on the sensing or recognition component. Molecular biosensors utilize enzymes, nucleic acids, receptors, aptamers, or antibodies as the recognition element (Iqbal *et al.,* 2000). Cell- and tissue-based biosensors utilize either a single type of cell or a collection of cells for both recognition and transduction in order to detect a biologically active analyte (DeBusschere and Kovacs, 2001). Common detection systems used to detect signals produced during the transductive step are physiologic, optic (bioluminescence), calorimetric (ELISAs), and electric (electrophysiology, action potentials, or changes in impedance).

The advantage of using a single whole cell (prokaryotic or eukaryotic) or a tissue-based system is that it has a natural selectivity for specific analytes. Therefore, it provides both physiologic and functional information on how a specific toxicant affects a living cell or tissue by making direct measurements of the relevant physiologic function. Examples of physiologic functions that can be monitored are: changes in metabolism, including pH (Baxter *et al.,* 1994; McConnell *et al.,* 1992; Parce *et al.,* 1989), oxygen consumption (Peters *et al.,* 1986), carbon dioxide production (Frahm *et al.,* 2002), redox potential (Gilardi and Fantuzzi, 2001), and lactate production (Cai *et al.,* 2002); electrophysiology, including membrane potentials (Glitsch, 2001) and action potentials (DeBusschere and Kovacs, 2001; Gilchrist *et al.,* 2001; Gross *et al.,* 1992, 1995; Pancrazio *et al.,* 1998); motility by ECIS/TAXIS (Hadjout *et al.,* 2001) and adhesion by ECIS (Giaever and Keese, 1984; Kowolenko *et al.*, 1990).

To date, much of the work on cell-based biosensors (CBB) has employed neurons (DeBusschere and Kovacs, 2001; Gilchrist *et al.,* 2001; Gross *et al.,* 1992, 1995; Pancrazio *et al.,* 1998). These devices have been designed to be portable detection systems for field analysis of possible environmental neuronal toxins and toxicants, including detection of chemical warfare agents. However, some of the major challenges in the development of a portable mammalian CBB are cell viability, shelf life, standardization, quality control, reproducibility, and cell stability during transportation (Gilchrist *et al.,* 2001). In order to maintain long-term cell viability, the medium must be refreshed periodically. Cell instability in the CBB is a concern due to mechanical vibrations and to any shear forces associated with media changes, which can dislodge the cells from the electrical sensing component. Kovacs was the first to describe a long-distance field trial using the HL-1 cell line, which was transported for several hours and remained functional (Gilchrist *et al.,* 2001).

One of the promising areas for biotechnologic fabrication and research is in the development of multi-component anal-

TABLE 1
Micro- and Nano-Biotechnologies for Toxicological Applications

Technology	Sensitivity	Toxicological application	Reference
Micropipet aspiration	Single cell	Effects on membrane viscoelasticity and function	Melder and Jain, 1994
Electric cell impedance sensor (ECIS)	Single cell	Effects on chemotaxis and cell attachment	Hadjout *et al.,* 2001; Kowolenko *et al.,* 1990
Traction force microscopy	Single focal adhesion	Effects on cell attachment and movement	Munevar *et al.,* 2001
Microelectromechanical systems (MEMs)	Single cell	Effects on phagocytosis and osmoregulation	Huang *et al.,* 2002; Lalan *et al.,* 2001; Polla *et al.,* 2000
Optical tweezers	Single cell and membrane ion channel	Effects on phagocytosis and membrane function	Kuo, 2001; Mehta *et al.,* 1999
Surface plasmon resonance (SPR)	FemtoMolar antigen	Effects on protein expression and cell function	Mozsolits and Aguilar, 2002; Von der Haar *et al.,* 2002
Grating coupled surface plasmon resonance (GC-SPR)	NanoMolar antigen	Effects on protein expression and cell function	Brockman and Fernandez, 2001
Laser capture microdissection	Single cell	Effects on cell and tissue function	Craven *et al.,* 2002
Atomic force microscopy	Single ligand-receptorinteraction	Effects on protein expression, membrane topography, and binding events	Dufrene, 2002; Allison *et al.,* 2002; da Silva, 2002
Microfabricated electrochemical detector array for exocytosis	Single vesicular release from single cell	Effects on vesicular trafficking and protein secretion	Dias *et al.,* 2002; Lacher *et al.,* 2001
Nanoelectromechanical systems (NEMs)	Monolayer of antibody and other proteins, single cell	Detection of pathogen presence and molecular interactions	Hess and Vogel, 2001; Ilic *et al.,* 2000, 2001
Biosensors			
Molecular		Detection of pathogen, toxicant, or biohazard presence	Cooper, 2002; Iqbal *et al.,* 2000
Whole cell		Detection of pathogen, toxicant, or biohazard presence	Aravanis *et al.,* 2001; Hall, 2002
Whole organ/tissue		Detection of pathogen, toxicant, or biohazard presence	Aravanis *et al.,* 2001; Jung *et al.,* 2001
Micro total analysis systems (μTAS)	Cellular and molecular interactions	Detection of pathogen, toxicant, or biohazard presence	Auroux, 2002; Khandurina and Guttman, 2002
Sentinel sensors	Bioterrorism detectors	Detection of pathogen, toxicant, or biohazard presence	Fu *et al.,* 2002; Mouradian, 2001

ysis microchips, such as micro total analysis systems (μTAS; Auroux *et al.,* 2002; Reyes *et al.,* 2002) or "lab-on-a-chip" devices (Fu *et al.,* 2002; Mouradian, 2001; Wang *et al.,* 2002) and "sentinel sensors." "Lab-on-a-chip" devices will contain all the necessary components (pumps, valves, detectors, etc.) to run an entire analysis, from sample preparation, through analysis to detection. This type of technology allows for the generation of smaller and faster devices as well as the scaling down of current tabletop laboratory equipment/technology into hand-held, field portable, or "point of care" devices (Knight, 2002). A representative example of this type of technology is the micro-fabricated fluorescence-activated cell sorter (μFACS), which has several advantages over conventional tabletop fluorescence-activated cell sorters (FACS) in that it is disposable, has higher sensitivity, and reduced background fluorescence. Other advantages include reduced opportunities for cross-contamination, exposure to bio-hazardous materials, and reduced cost. These μFACS devices can be used as a stand-alone device or as a front-end device for a μTAS (Fu *et al.,* 1999, 2002).

From an environmental or public health perspective, field portable devices, which rapidly detect and identify toxicants in the environment, are of vital importance. Industrial waste products which have been buried, released in streams and rivers, or released into the atmosphere and the current threat of chemical and biologic weaponry make the early detection of environmental toxicants, nerve agents, biological toxins and pathogens critical for rapid diagnosis. "Sentinel sensors" or "animal-on-a-chip" devices would be able to sense pathogenic concentrations of gases and environmental toxicants as a first-line warning device. Mammalian and nonmammalian species, otherwise termed "sentinel species," have been used for centuries as early warning detectors to protect humans against lethal concentrations of gases and environmental toxicants. A device that could identify potential problem areas before they seriously affect and animal species would be very important in the preservation of our environment.

The micro- and nano-fabricated devices described in this review only represent a small fraction of this rapidly growing toolbox available to the toxicologist. Table 1 summarizes some

of the technologies described in this review and suggests some potential toxicological uses for these procedures. These technologies are useful in the research lab and soon will prompt dramatic changes in the health care arena. Some of these changes will result in the ability of a physician to rapidly screen and diagnose a patient from a drop of blood on a disposable microarray chip designed to detect toxicants, changes in biochemical parameters, infection with a particular bacterial or viral organism, or quantification of lymphocyte subtypes, as a diagnostic test for HIV infection progressing to AIDS. These diagnostic tests will be done in the field rather than a remote laboratory, thereby decreasing the turnover time for analysis enabling prompt treatment of the patient. Subsequent drug treatment would utilize biodegradable micro- or nano-drug delivery systems, which will slowly deliver drugs and selectively target drug delivery, which will reduce daily systemic medications (Chandy *et al.,* 2001; Dass and Su, 2001; Prabhu *et al.,* 2002). Implantable sensors and probes will be available for a number of diseases, such as diabetes and Parkinson's disease, respectively. The implantable sensors will be able to detect changes in biochemical parameters such as glucose levels in real time and automatically administer the appropriate dose of insulin from an external insulin pump. This technology will eliminate multiple daily finger or forearm blood sticks and will allow for better control and regulation over diabetes. Implantable probes or prostheses could be used to stimulate nerves in paralysis patients and regions of the brain, such as the substantia nigra, to relieve the physical symptoms of Parkinson's disease. Similar devices, such as cochlear prosthetic implants, have already been used with remarkable success. Many surgical procedures already use micro-fabricated tools and instruments, which has allowed for quicker and less evasive surgical procedures. This directly relates to decreased trauma to the patient, decreased healing time, smaller scars, same day procedures, less overnight hospital stays, and therefore decreased hospital costs. However, this is only the beginning for the use of micro- and nano-fabricated devices for surgery. With the development of laser tweezers and multiphoton microscopes, the ability to do dissection and surgery on a single cell "nanosurgery" to remove harmful genes may soon be a reality (Konig *et al.,* 1999; Tirlapur and Konig, 2002). Two-photon microscopy may also allow for *in vivo* monitoring of leukocyte trafficking (Miller *et al.,* 2002) and specific cellular interactions at the molecular level in real time (Bousso *et al.,* 2002). Micro- and nano-fabrication and technology has and will change for the better the way we perform toxicological assessments in the research lab and the diagnosis and treatment of patients. The science fiction world of microrobots and probes, which can circulate in the blood stream and specifically seek out and repair lesions, remove plaques from arteries or to specifically target and destroy cancer cells may be just over the horizon.

ACKNOWLEDGMENTS

This work was supported, in part, by the Nanobiotechnology Center (NBTC), a STC Program of the National Science Foundation under Agreement No. ECS-9876771 (D.A.L.), and grants to M.A.L. (ES07408, EB00208, NAS9-02016).

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