Perspective

The Rapidly Growing Field of Micro and Nanotechnology to Measure Living Cells

Adrienne R. Minerick
Dave C. Swalm School of Chemical Engineering, Mississippi State University, 323 President's Circle, Box 9595, Mississippi State, MS 39762

DOI 10.1002/aic.11615
Published online July 28, 2008 in Wiley InterScience (www.interscience.wiley.com).

Keywords: microfluidics, nanotechnology, red blood cells, lab on a chip, microdevice

Introduction

Living cells are complicated bioreactors with a plethora of multistage reaction sequences all occurring in concert to sustain the factory of life. As chemical engineers develop more sophisticated nanostructures and microdevices in coordination with electrical engineers and chemists, the ability to measure and monitor these cellular and subcellular processes is becoming a reality. Traditional cell analysis tools utilized over the last 25 years have predominantly been conducted at the macroscale. However, the trailing technology of the Silicon Revolution has been microfabricated laboratories on a chip (Lab-on-a-Chip), with the ability to interface micron-scale events and sensors with cell populations. The infusion of nanotechnology into this platform is even more recent and began after the National Nanotechnology Initiative's (NNI) flurry of research in this area. Today, micro and nanoscale technologies are routinely used to probe and manipulate living cells. Although a few platforms are currently available on consumer markets, the potential for economic growth is significant. This perspective focuses primarily on the progress made utilizing microscale devices and nanotechnology to probe living cells for applications as diverse as medical diagnostics, pathogen or bioterrorism detection, pharmaceutical screening and cancer detection.

Microdevices (also called Lab-on-a-Chip (LOC), and micro Total Analytical Systems (μTAS)) are under development in labs across the globe because they have the potential to provide high-resolution, low-cost, and rapid analysis with small sample volumes for a wide range of biological and chemical applications. Such microdevices are designed to mimic laboratory processes in micro and nanoliter volumes by utilizing microchannels and microchambers fabricated into polymer or glass chips. Living cells can be studied within this platform as precisely controlled environmental conditions and single-cell manipulations are now possible.[1]

Correspondence concerning this article should be addressed to A. R. Minerick at minerick@che.msstate.edu.

Most cell manipulation technology takes advantage of the significantly different probability of object interactions that occur at the microscale. For example, examining a 10 cm section of an approximately 4 in. dia. (100 mm) pipe, one finds that the inner surface area of the pipe is 0.03 m^2 with a corresponding volume of 0.0008 m^3. The surface to volume ratio for this large channel is only about 40 m^{-1}, while for a microchannel with a diameter of 100 microns and the same 10 cm long, the surface area and volume are three and six orders of magnitude smaller, respectively, but the ratio of surface area to volume is three orders of magnitude larger (=40,000 m^{-1}). This trend is briefly captured in Table 1 for spherical particles, as well as for channels. This means that liquid/solid interactions are much more prevalent, and as such, the surfaces can be utilized to impart forces on the fluid and the cells within the channel.

This surface to volume attribute can be exploited further when moving three orders of magnitude smaller to nanoparticles. For instance, one can coat a 1-micron spherical cell with over 3 million 1 nm particles (no void space = 4 million). Functionalizing a nanoparticle is fairly straightforward, as well because over 300 1-Angstrom molecules can fit on a 1 nm particle (no void space = 400). Molecules that are used to functionalize surfaces require a high affinity and specificity for their target molecules. Antibodies, a highly specialized biological (immunological) recognition tag, are the most commonly utilized molecules for these purposes. In this perspective, discussions will focus on microfluidic device technologies for probing cellular level processes, molecular influences on cell responses, and cell to nanoparticle interactions.

Microfluidic platforms capable of manipulating single-cells

The ability to probe cells at the length-scale of single molecule is steadily becoming a reality, such that recognition of biological or mechanical dysfunction in a few cells of an entire population will soon be possible. This ability would enable highly sensitive tools for detecting cancer, assessing the effectiveness of pharmaceutical treatments, and quantifying biolog-

Table 1. Surface to Volume Ratios Particles and for 10 cm Long Channels

Spherical Particle		Square or Cylindrical Channel (10 cm long)	
Diameter	S / V Ratio (1/m)	W=H or Diameter	S / V Ratio (1/m)
1 mm	6.0 E+03	100 mm	4.0 E+01
1 μm	6.0 E+06	100 μm	4.0 E+04
1 nm	6.0 E+09	100 nm	4.0 E+07

Particles and structures at the nanoscale have much great S/V ratios that impart new functionalities

ical or chemical toxins. While nanoscale tools are needed for spatial and temporal cell probing, microfluidic platforms are required to transport and manipulate the cells, such that they can be interfaced with the appropriate nanoprobe. The microfluidic systems will need to be fully interfaced for nanoprobe to detector transfer of information. This section discusses the fabrication of such microdevices, the functionality of components of the lab-on-a-chip devices, and then discusses the electrical, magnetic, optical, and mechanical forces utilized to manipulate living cells within the devices.

The Silicon Revolution developed technology that has provided the foundation for much of the microfluidic devices utilized today. Fabrication techniques for micron and nanoscale features are diverse and include micromaching masters,[2] (soft) photolithography,[3] injection molding, glass acid etching, reactive ion etching, laser ablation,[4] shrinky dink, hydrophobic surface patterning,[5] paper-based microfluidics,[6] masked electrodeposition, microprinting, masked growth, LiGA (combines lithography, electroplating, and molding), and many others.[7] Polymers are the most common platform for microfluidic devices due to the ease of channel fabrication, their tunable chemical, mechanical, electrical, and optical properties, as well as lower expense of the material.[8] Micromachined metal mold masters are viable for mass-producing hot embossed thermoplastic devices with an inverted pattern of the master. One such thermoplastic, poly(methyl methacrylate) (PMMA), has material properties such as surface charge or optical characteristics that are conducive to electroosmotic flow in the channels and unimpeded spectrometric detection. The master is a considerable expense, but almost 1 million plastic devices can be embossed from each master, thus, making the cost per device quite low.[2] However, photolithographic fabrication techniques are more conducive to research laboratories because of rapid turnaround time of prototypes. A monomer containing eight epoxy resins known as SU-8, can be spin-coated onto silicon wafers, masked with high-resolution printed photomask film, and developed to produce features as small as 0.5 microns.

Poly(dimethylsiloxane) (PDMS) elastomers can then be cast onto this master to produce a relatively flexible inverse casting of the original master. Cell culture, cell population analysis, and single-cell manipulation has been accomplished in devices fabricated from the aforementioned methods.[1–3]

In order for microfluidic devices to achieve the goal of fully inclusive tiny laboratories on a chip, they must mimic multistep analytical procedures with nanoliter volumes of reagents and samples. For the devices to be portable, this must be accomplishable in nonlaboratory conditions with portable power sources and detection schemes. From a chemical engineering perspective, it is necessary to re-engineer key unit operations at the microscale by taking advantage of forces that are much more significant at these small length-scales. Necessary unit operations include transport, dilution, mixing, thermal control, concentration, separation, and detection. One of the first discoveries with microfluidic devices was that as channel dimensions are decreased, the larger surface areas with respect to volume creates significant drag along the channel walls, and the subsequent pressure drop down a channel is significant. Also, the classic parabolic velocity profile results in significant dispersion in the channel, which depending on the application, is not desired. Further, pressure driven flow is less amenable to miniaturization, but has been accomplished on chips with creative solutions that range from peristaltic pumps driven from electrical actuators to centrifugal pumps.[9] Electroendoosmotic flow (EOF) is an alternative and is accomplished with longitudinal electric fields that exert forces on double layer charges near the channel walls. Through viscous shearing, relatively high electric fields ($\approx$100 V/cm) can cause convective motion of the bulk fluid in the channel, and a flat velocity profile is produced such that hydrodynamic dispersion is minimized in the flow. Disadvantages are that the large voltages can cause Joule heating within the fluid, as well as electrochemical reactions at electrode surfaces, and, thus, impact ion gradients within the channels.[10] Although pressure driven and electrokinetic driven transport are the most prevalent, transport strategies also include surface tension biases,[5] thermal gradients, magnetic, geometric designs, and gravity among others. It has been advanced that flow driven by forces generated inside flow channels that take advantage of micro/nanoscale surface characteristics will be most effective.[9]

Chemical manipulation and analysis is sometimes easier in microfluidic channels than whole cell characterizations, because living cells can dynamically respond to and even change their surrounding environment. This can be quite significant in nanoliter reaction chambers and channels, because even migration of a small number of ions can effect local ion concentrations due to the finite nature of the system. The ions in solutions dynamically interact with channel walls, especially in electroendosmotic flow, and transport in the microfluidic device can be altered.[10] Despite these challenges, progress has been made in controlling transport of reagents, capturing, and concentrating cells in microfluidic devices.[1]

Mixing is a challenging area that has been explored by many researchers. At the length scales of interest, mixing by molecular diffusion alone is very time intensive; diffusional timescales across a microchannel can take 15 min.[11] The mixing of living cells adds complication because the cells generally migrate to the central axis of pressure drive flow due to the hydrodynamic shear effects near the wall.[12] However, mixing of reagents can be accomplished with a variety of techniques including creative geometries, intentional formation of vortices, and oscillatory pumping.[11]

The different mechanisms for manipulation and analysis of living cells include specialized cell culture, cell concentration, cell lysis, and separation.[1] A recent example is specially fabricated micro and nanopatterned gold surfaces functionalized with fibronectin for adhesion. These structures were then embedded in microfluidic channels to capture cells for subsequent culture.[13] One unique application required precisely

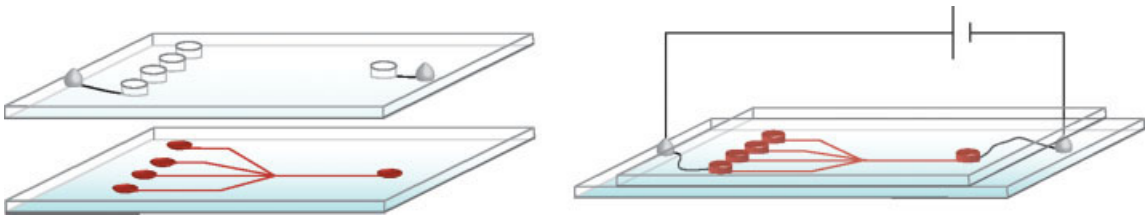

Figure 1. Second-generation microdevice with one loading port and four exit ports for each of the four positive blood types.
ABO antigens influence deflection in the device's dielectrophoretic field causing the cells to flow into different channels for subsequent optical detection (adapted from[19], graphic drawn by Heather Rowe, MSU).

controlled multicellular tumor spheroids grown in a microfluidic array assembly comprised of U-shaped traps made of PDMS. Seed tumor cells were introduced into the array, single-cells became immobilized in each trap, and the microfluidic platform was then used to perfuse nutrients for cell development into spheroids whose properties mimic those of tumors *in vivo*. Once the spheroid tumors were grown, this platform enabled streamlined screening of cancer drugs.[14] Additional cell manipulations have been demonstrated including microdevice cell lysis by heat generation near the cell membrane by a laser irradiated magnetic bead system (LIMB),[15] DC electroporation, and contact with caustic lytic agents that dissolve the membrane.[1] Cell separation can be accomplished in a number of ways including size fractionation, charge and conductivity differences, centrifugal and other mechanical forces, magnetic bead assisted separations, density, and laser and optical forces.[1] Dielectrophoresis (nonuniform AC fields) is frequently used for cell separations by either size or cell polarizability, and has been shown to distinguish Malarial infected cells from healthy cells and live from dead cells at throughputs on the order of 10^4 cells/second.[16,17] The plethora of tools available to accomplish unit operations within these microfluidic lab-on-a-chip devices are quite diverse; the most promising utilize technologies which can be scaled down to be fully housed on the chip and, are, thus, portable instruments for use in remote locations.

Discerning molecular differences between cells

Not only are electrical and magnetic fields convenient from a portability standpoint, recent findings suggest that these tools have the sensitivity to discern differences in molecular expression on the same type of cells. This year, our group reported the ability to discern positive blood types without significant blood sample pretreatment, without molecular modification, and with only a 6 Volt peak to peak AC dielectrophoretic field.[18] This work was accomplished within the confines of a custom built microdevice, and a field applied from an external function generator. The second-generation device is depicted in Figure 1.[19]

Human blood samples are rather convenient from both a medical diagnostic, as well as a cell population standpoint. Humans have differing molecular structure of blood type antigens expressed on the surface of red blood cells. There are 22 blood group antigen classification systems, and 6 antigen collections in the process of being placed into a system. The most prevalent and important system is the ABO blood type, because it determines blood transfusion compatibility; it is classified by type based on antigen polysaccharides expressed on the membrane surface and antibodies circulating free in blood plasma.[20] Type A blood expresses antigen A and a powerful IgM antibody Anti-B, while Type B blood cells express antigen B and IgM antibody Anti-A. Type AB blood carries both A and B antigens and no antibodies, while Type O blood has a nonfunctionalized backbone antigen and expresses both IgM antibodies. As a result, a Type O person is considered a universal donor, while a Type AB person is considered a universal acceptor. However, one more antigen, the Rhesus factor, is key to track for blood donations. If the Rh antigen is present, a blood type is referred to as positive; if absent, the blood type is negative. The resulting eight blood types in the ABO/Rh system are A+, B+, AB+, O+, A−, B−, AB−, and O−. Human A and B blood group antigens differ from each other by only a substitution on the terminal end of the H antigen as shown in Figure 2.[20] The number of ABO antigens per cell varies by individual, but can be as high as 1.5 million,[21] so while the molecular structure differs only slightly, a sizable percentage of the surface area of an 8-micron red blood cell is dedicated to expressing the ABO antigens.

Red blood cells experience complex interactions with electric fields due in part to their intra- and extra-cellular composition, dynamically responsive functionality, and unique biconcave shape. Cell membranes are essentially nonconducting ($\sigma \leq 1$ μS/m),[16] vary from 90 nm to 50 nm in thickness,[22] and impact the ability of charges to penetrate the cell, thus, making the cell a dielectric object in solution.[17] Intracellular charged proteins, hemoglobin, and cytosol molecules contribute to an erythrocytes' high interior conductivity ($\sigma = 0.53$ to 0.31 S/m),[23,16] and impact the ability of the cell to conduct charges through it.[17]

In our laboratory, whole blood was diluted 600:1 with phosphate buffer saline, and this blood cell suspension was observed while an alternating current, spatially nonuniform field was applied in the sample chamber. Figure 3 shows the unaltered video microscopy images of the nonuniform electrode configuration and the spatial distribution of A+, B+, AB+ and O+ blood cells in the microdevice chamber.[18] Image analysis to determine cell centroid x,y positions was conducted on the video microscopy sequences. The average cell position was then compiled in MATLAB and tracked with time with a wedge binning approach that approximated the electric field gradient. O+ erythrocytes exhibited the smallest movement in the dielectrophoretic field likely due to the presence of only a nonfunctional antigen backbone

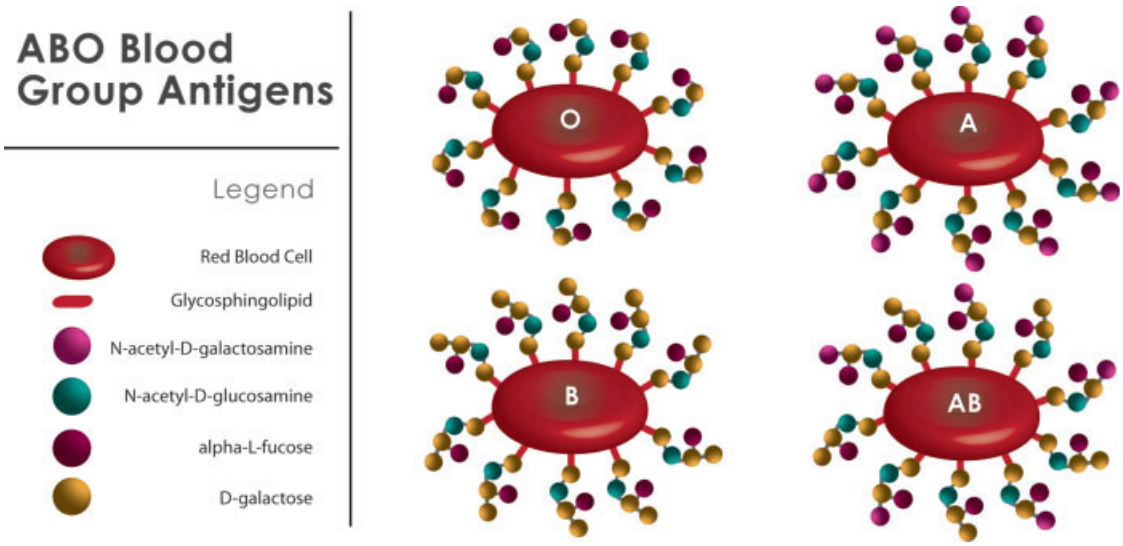

Figure 2. ABO blood group antigens shown with each of the four base blood types.
An additional antigen, Rh factor (not shown), determines the positive and negative blood types in the ABO system (graphic drawn by Heather Rowe, MSU).

(Figure 2). A+ cells responded to the field by moving in the vertical direction away from the high-field density electrode region, while B+ cells responded by moving horizontally out from both electrodes. This is illustrated qualitatively in Figure 3. AB+ average responses showed slightly greater horizontal movement than O+, and slightly greater vertical movement than B+. Our group has attributed this behavior to the differing dielectrophoretic forces imparted by the A and B glycoforms on AB blood cells. While these cells express structurally similar antigens that differ only by their terminal saccharides,[20] statistical analysis revealed that we could recognize O+ blood with greater than 95% confidence. This has large implications because type O blood can be universally donated. AB+ could be distinguished with greater than 85% confi-

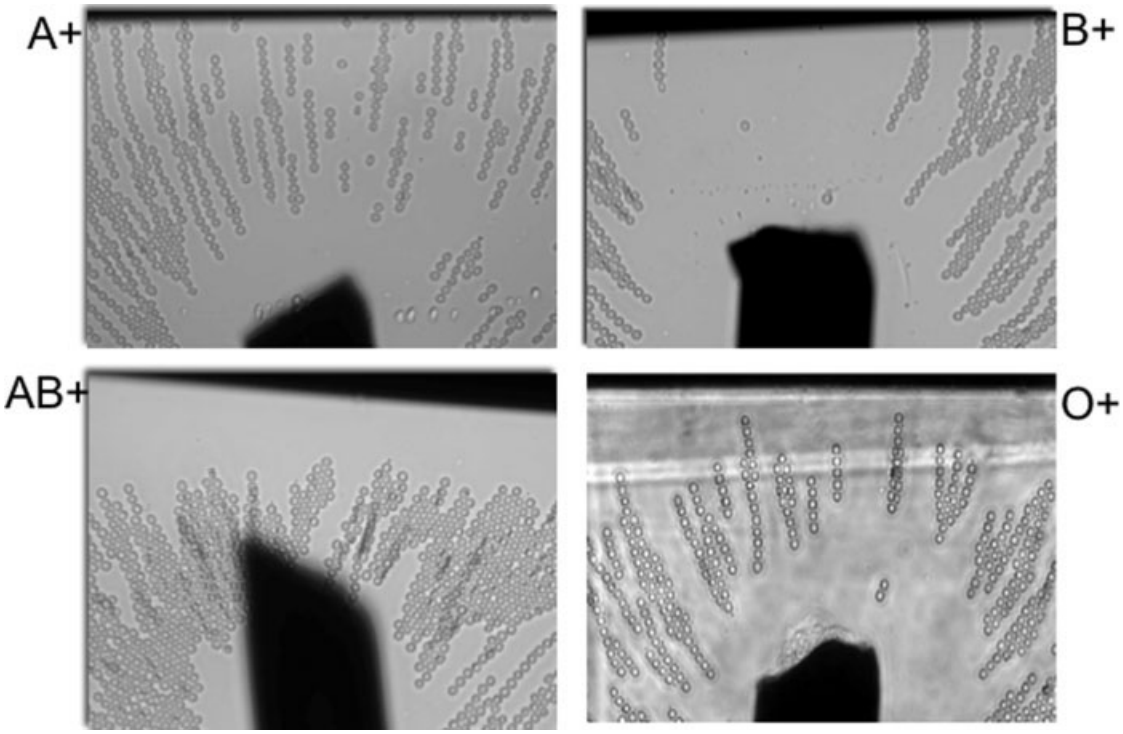

Figure 3. Unaltered images of the four positive ABO blood type cells in a dielectrophoretic field.
Differing responses in the vertical and horizontal dimensions are quantified for each blood type such that O+ blood can be distinguished at greater than 95% confidence.

dence, and all blood types could be distinguished from each other at a 56% confidence level.[18] With our second-generation microdevice (Figure 1), and by engineering antibody-linked nanoparticles to bind to these antigens, we expect to increase these confidence levels significantly.

Nanotechnology used to probe single-cells

Nanotechnology for mechanical, biochemical, and electrical probing of living cells and subcellular level events is diverse. This section discusses single particle interactions with cells that influence or change cell properties for greater contrast, optical tagging, changes in susceptibility to forces, or even direct penetration of the cell.[24] Structures with nanoscale features can play a significant role in manipulation and characterization of living cells. Also, nanoscaffolds can provide a framework for cell growth and the inclusion of nanoscale materials into tissue can have a significant impact on tissue properties, regeneration, strength, and controlled release of drugs.

While nanoscale structures exhibit different properties than their bulk counterparts, many nanoparticles utilized with living cells are functionalized with antibodies (cover figure), or other biological molecules exhibiting high-levels of specificity and selectivity. These antibody interactions have been previously characterized by immunologists, cloned, amplified within animals or *in vitro*, harvested and then purified. For most nanoparticle binding, a linker molecule is required such as mercaptopropyltriethoxysilane's (MPTS) affinity for gold nanoparticles, and the exposed amine group's affinity for the desired antibody.

Noninvasive imaging of living cells or tissues is possible with nanoparticles or quantum dots as contrast agents; they can be incorporated into the cells via endocytotic mechanisms or simply linked onto the cell surfaces.[25] Traditional organic fluorochromes are limited to yield resolutions of approximately half the wavelength of light delivered to the sample ($\approx$300 nm) and the molecules themselves are susceptible to optical degradation (photobleaching), and have wide emission bands such that it is difficult to use more than three colored fluorochromes at one time.[26] However, gold nanoparticles, quantum dots, and superparamagnetic particles are not susceptible to these issues. Gold can be functionalized with antibodies, has proven to be biocompatible, and is an excellent contrast agent in optical and electron microscopes for structures as small as macromolecules.[25] Approximately 100 to 10,000 metal and semiconductor atoms are arranged together to form a quantum dot, which has unparalleled capabilities as fluorescent probes for high-resolution imaging with high-intensity laser light sources. Quantum dots are amenable to cell labeling within microdevices, but due to the base materials of cadmium and arsenic, these are not conducive to *in vivo* imaging.[25]

Nanoparticles can also be bound to cells for subsequent magnetic or electrical manipulation. One example of this is with superparamagnetic beads bound to transmembrane receptors of target cells with monovalent ligands. When in a magnetic field, the beads became magnetized and aggregated the cells together. Due to the proximity of the transmembrane receptors, biological responses such as calcium influx into the cell occurred only when exposed to the field.[27] Magnetic glyco-nanoparticles comprised of Fe_3O_4 coated with silica, D-mannose, and a fluorescein molecule for imaging were able to bind to *E. coli*, and then were concentrated and pulled out of solution when a magnetic field was applied.[28]

Other ligands have an affinity for membrane proteins and can aid in cell adhesion to specific areas of a microdevice channel or nanoprobe. Functionalized fiber optics have been used to image single cells. A target analyte from the cell interacts via immunochemical complexation with antibodies bound to the tip of a fiber optic thread. This is converted to an electrical signal and then reconstituted into an image of the single-cell. By coupling this technology with a fluorochrome, nanoresolution enzyme linked immunosorbent assays (ELISA) can be realized. To date, this work has been predominantly used to monitor programmed cell death (apoptosis).[25] Once immobilized, other mechanical probing techniques can be used on the living cell such as atomic force microscopy. In one study, AFM was used to measure the stiffness of live metastatic cancer cells as compared to healthy cells. It was determined that metastatic cancer cells are 70% softer than the benign cells.[29]

Biological cells can also be captured into specialized cages or impaled with nanofibers to aid in still imaging of the living cells. In order to create nanoaquariums, femtosecond laser pulses were used to create hollow microstructures inside a photostructural glass microdevice. Large single celled organisms with dimensions of 100 μm by 40 μm were imaged inside the confines of the nanoaquarium.[4] This confinement improves the ability of light microscopes to image the organism. Complete immobilization for longer-term observation of cells has also been attempted via impaling or cell culture on carbon nanofibers, or silicon nanoneedle arrays on surfaces.[25] Vertically aligned carbon nanofibers (VANCF) are cones or cylinders grown on silicon surfaces whose dimensions are as small as 10 nm at the tip, and as long as 10 microns in height. They can be grown in regular arrays on surfaces, are extremely strong owing to their graphene sheet composition, can be integrated with microscale features in devices, and can have their tip functionalized for electrochemical studies. These tools have been used to probe cells by either impaling via centrifugation or via mammalian cell culture on the cones. DNA can be bound to the nanofiber, introduced into the cells via penetration, and the cells have been demonstrated to live several weeks and express the new DNA.[25]

Nanofibrous scaffolds have also been used to mimic the composition and structure of a cell's native extracellular matrix (ECM).[25] Some tissue scaffolds can be assembled *in situ* in living tissue. One example of this is peptide amphiphile molecules that assemble into supramolecular nanofibers in the ionic environment of the spinal cord. The molecules self-assemble into cylindrical nanofibers exhibiting a necessary laminin epitope, a molecular structure recognized by the immune system, that promotes neural stem cell growth to repair motor and sensory fibers.[30] Further, carbon nanotubes (CNT) are being used in synthetic ECMs because the CNT size and structure is similar to native ECM proteins. The roughness of the CNTs, as well as an applied electrical stimulation through the conductive nanotubes was found to increase cartilage regeneration.[31]

There exist a multitude of nanointerfaces available to probe living cells both *in vivo* and *in vitro* on a microfluidic chip. Challenges lie in seamlessly integrating techniques into a robust and versatile, yet reliable, platform with enough throughput that single-cell events can be compiled into profiles of the cell population. The applications of this are widespread in

both diagnostics, pharmaceutical screening, and even furthering our knowledge of subcellular events.

Health and Environmental Concerns Regarding Micro- and Nanotools

Our pursuit of chemistry and related knowledge has not always occurred under the safest or environmentally benign conditions. The most common approach has always been to single-mindedly pursue objectives. The most famous example is of Nobel laureate, Marie Curie, and her husband's efforts in discovering and isolating radium. The research was done without any protection or containment practices because the damaging effects of ionizing radiation were unknown at that time.

However, none of us, as avid life-long learners and open minded researchers, should be so naive as to think that the high-surface area nanostructures that we are synthesizing could be benign from either a health, safety, or environmental perspective simply because the more traditional macroscale form of the elements is inert.[32]

In the opinion of this author, supplemental funding should be included from any agency participating in the National Nanotechnology Initiative's broad goal; such funding would be available for each PI's research program to concurrently conduct preliminary health and environmental screenings and publish these results in the open literature. This could be a chapter in each student's thesis, and would train our upcoming workforce to be well versed, proficient, and cognizant of toxicity screening techniques, as well as adverse chemical interactions. Concerns regarding the reliability of such data would be resolved in the literature arena, where the results first undergo peer-review, and then subsequently undergo scrutiny in the broader research community. Independent agencies could continue to conduct their independent toxicology studies, but possibly would be in a position to conduct tests on research grade materials instead of on materials nearing release into the greater consumer markets.

One recent example of this is the use of silver nanoparticles as antimicrobial agents in a multitude of products from plastic ware to socks that are odor resistant. Such products have been on the market for over 5 years, yet "a complete characterization of the toxicity of environmentally relevant silver species is lacking, only a limited risk assessment is possible at this time" (2008).[33] Recent evidence suggests that nanoparticle embedded textiles leach as much as 650 μg with each washing, suggesting that these materials are mobilized in the wastewater run-off system rather rapidly.[34] The ability to complete toxicity characterization and risk assessments could be completed in the research phase, long before products reach the market. As evidenced by Marie Curie's noble career and recent news regarding silver nanoparticles, we should not use the world's ecosystem as our experimental conditions. We have the means to conduct this assessment[32] concurrent with our research and will do more to advance the potential of micro and nanotechnology with this approach than by continuing the paradigm of trial and error.

Existing Challenges and Future Goals

While some political challenges exist with coordinating health and environmental assessments in this field, existing technical challenges are extensive. Progress on this front is rapid; new particles, new functionalized nanoplatforms, nanocomposite materials, and creative new approaches to utilizing the dominating forces at these scales are being developed everyday. Some viable pathways are briefly discussed here.

While microfluidic devices have evolved to include precise manipulations of small volumes and single cells, few fully integrated, self-contained, sample preparation to final analysis devices currently exist. Systems that utilize disposable chips with millifluidic channels and reaction chambers that interface with a completely contained benchtop sensor instrument are currently on the market. One notable example in blood sample testing is the Piccola Xpress from Abaxis® that can complete a standard metabolic panel with 0.1 mL of whole blood in about 12 min.[35] This system includes an advanced self-calibration and quality control scheme for simultaneous control runs, checks for false results due to chip malfunction, monitors noise associated with the sensor, electrical board, and wavelength detection. There is considerable room in this market for portable, hand-held, field-ready analytical and diagnostic devices requiring less volume, less time, and less money.

Nanocomposite patterned surfaces have yet to be integrated with microfluidic devices. These two research approaches are evolving separately, but once the structured growth of nanostructures is streamlined, these two platforms will be combined and will likely offer the next big advance in medical diagnostics, pharmaceutical screening, or pathogen detection. Nanopatterned surfaces within microchannels will likely enable precise electrokinetic control of the fluid, as well as a fully wired microarray of nanosensors.

A wide variety of smart polymers are being developed that respond via large conformational changes to stimuli in their surrounding environments. These polymers, if embedded in microfluidic devices would enable new flow, separation, and other functionalities in a dynamically controlled micro/nano lab-on-a-chip network. The technology is not yet at this stage, but it is currently at a state where polymers will swell or contract to control pore size in nanoporous silicon-based membranes.[36]

Microplexing via planar array or suspension array technology are extremely valuable tools that have significantly advanced genomic and proteomic screenings, and are pervasively used for molecular detection from cell lysates or as assays for pathogen detection.[37] Combinations of complementary receptor tags are patterned and bound onto a surface with planar arrays; the target then binds only to its specific tag. However, the disadvantage of this platform is the inherently long diffusion time for the target to be intercepted by the immobilized tag, which unacceptably slows test results when the target is present in low concentrations. Microfluidics is a viable platform to facilitate mass transfer of the target over the fixed receptor regions, and through integration with electric field concentration strategies[38] has the potential to overcome concentration challenges.

Another recent approach has been to mobilize the microarray receptors. Two exciting examples exemplify this approach. Mobile barcoded microparticles can not only overcome mass transport challenges, but also overcome unique fluorescent spectra limitations. When a detection tag is mobile, each tag then requires a specific fluorophore emission spectrum. Unfortunately, the detectable spectrum of light only allows for about 100 unique spectral signals, which is not sufficient for most

multiplexing applications and is expensive.[37] One solution was to develop spatial resolution within a mobile 300-micron particle, where one side was blank to allow for pattern orientation with the detector, and the second side was dot-coded with a fluorophore in a spatial scheme allowing for over 1 million unique patterns. As proof of concept, these particles were used in a millifluidic platform for detection of two 20 bp DNA oligonucleotides.[37] The second approach has been to develop multistriped metal nanowires that have been functionalized for immunoassay detection of biowarfare agents or DNA hybridization assays.[39] The potential exists in both of these platforms to perform true multiplexing with greater than 100,000 targets.

Also, the use of immobilized coatings and nanoparticles, which are not released into the surrounding environment is attractive, but require efficient contact of the target with the surface. Maintaining sterilization of hospital equipment is one scenario where this approach is highly desirable. Permanent microbicidal coatings are being developed that disrupt microbial membranes, but leave mammalian cells untouched.[40]

The future will see even more creative strategies to study and manipulate living cells that rely on both microfluidics and nanoscale probes. Chemical engineers who are proficient across many disciplines will continue to be essential in advancing technology development, as well as describing and exploiting fundamental forces at the micro and nanoscale. Microanalytical lab-on-a-chip devices are the key platform that will enable single-cell analysis and impact medical diagnostics, biowarfare detection, pharmaceutical testing, and many other chemical analysis applications. It is expected that these platforms will continue to grow and will see widespread use on the consumer markets in the next 10 years.

Acknowledgments

The author gratefully acknowledges the figure artwork by Heather Rowe. Further, appreciation and acknowledgment is extended to the current and past members of her research group who are, in reverse chronological order, Kaela M. Leonard (current Ph.D. student), Soumya S. Keshavamurthy-Srivastava (current Ph.D. student), Aytug Gencoglu (current Ph.D. student), Eric Beauregard (past Ph.D. student), Prashant Daggolu (past M.S.), and Kellie M. Smith (past M.S. student). Appreciation is also extended for the training and guidance of Dr. Hsueh-Chia Chang at the University of Notre Dame.[24] This work was supported in part by an NSF CAREER (CBET 0644538) and the Oak Ridge Associated Universities Ralph Powe Junior Faculty Awards.

Notation

μTAS = micro-total analysis systems
CNT = carbon nanotubes
DEP = dielectrophoresis
ECM = extra cellular matrix
EOF = electroosmotic flow
LOC = lab-on-a-chip
NNI = National Nanotechnology Initiative

Literature Cited

1. Yi CQ, Li CW, Ji SL, Yang MS. Microfluidics technology for manipulation and analysis of biological cells. *Anal Chim Acta*. 2006;560:1–2,1–23.
2. Hupert, ML, Guy WJ,. Llopis SD, Shadpour H, Rani S, Nikitopoulos DE, Soper SA. Evaluation of micromilled metal mold masters for the replication of microchip electrophoresis devices. *Microfluid Nanofluid*. 2007;3:1–11.
3. Anderson JR, Chiu DT, Jackman RJ, Chernlavskaya O, McDonald JC, Wu HK, Whitesides SH, Whitesides GM. Fabrication of topologically complex three-dimensional microfluidic systems in PDMS by rapid prototyping. *Analytic Chem*. 2000;72:14,3158–3164.
4. Hanada Y, Sugioka K, Kawano H, Ishikawa IS, Miyawaki A, Midorikawa K. Nano-aquarium for dynamic observation of living cells fabricated by femtosecond laser direct writing of photostructurable glass. *Biomed Microdevices*. 2008;10:3,403–410.
5. Cho SK, Moon HJ, Kim CJ. Creating, transporting, cutting, and merging liquid droplets by electrowetting-based actuation for digital microfluidic circuits. *J Microelectromech Syst*. 2003;12:1,70–80.
6. Martinez AW, Phillips ST, Carrilho E, Thomas SW, Sindi H, Whitesides GM. Simple telemedicine for developing regions: Camera phones and paper-based microfluidic devices for real-time, off-site diagnosis. *Anal. Chem.* 2008;80:10,3699–3707.
7. Gates BD, Xu QB, Stewart M, Ryan D, Willson CG, Whitesides GM. New approaches to nanofabrication: Molding, printing, and other techniques. *Chem Rev*. 2005;105:4,1171–1196.
8. Becker H, Gartner C. Polymer microfabrication technologies for microfluidic systems. *Anal. Bioanal Chem.* 2008; 390:1,89–111.
9. Pennathur S. Flow control in microfluidics: are the workhorse flows adequate? *Lab Chip*. 2008;8:3,383–387.
10. Minerick AR, Ostafin A, Chang H.-C. Electrokinetic transport of red blood cells in Micro-capillaries. *Electrophoresis*. 2002;23:2165–2173.
11. Ottino JM, Wiggens S. Introduction: mixing in microfluidics. *Philos Trans R Soc London, Ser A*. 2004;362: 923–935.
12. Munn LL, Dupin MM. Blood cell interactions and segregation in flow. *Anal Biomed Eng*. 2008;36:4,534–544.
13. Goto M, Tsukahara T, Sato K, Kitamori T. Micro- and nanometer-scale patterned surface in a microchannel for cell culture in microfluidic devices. *Anal Bioanal Chem.* 2008;390:817–823.
14. Wu LY, Di Carlo D, Lee LP. Microfluidic self-assembly of tumor spheroids for anticancer drug discovery. *Biomed Microdevices*. 2008;10:2,197–202.
15. Cho YK, Lee JG, Park JM, Lee BS, Lee Y, Ko C. One-step pathogen specific DNA extraction from whole blood on a centrifugal microfluidic device. *Lab Chip*. 2007;7:5, 565–573.
16. Gascoyne P, Mahidol C, Ruchirawat M, Satayavivad J, Watcharasit P, Becker FF. Microsample preparation by dielectrophoresis: isolation of malaria. *Lab Chip*. 2002;2,70–75.
17. Pethig R. Dielectrophoresis: Using Inhomogeneous AC electric fields to separate and manipulate cells. *Crit Rev Biotechnol*. 1996;16:4,331–348.
18. Keshavamurthy SS, Daggolu PR, Burgess SC, Minerick AR. Dielectrophoretic characterization of erythrocytes:

Positive ABO blood types. *Electrophoresis*, in press, 2008.
19. Keshavamurthy SS. Leonard KM, Burgess SC, Minerick AM. Direct current dielectrophoretic characterization of erythrocytes: Positive ABO blood types. *NSTI-nanotech*. 2008;2,401–404.
20. Patenaude S, NOL Seto, Borisova SN, A Szpacenko A, Marcus SL, Palcic MM, Evans SV. The structural basis for specificity in human ABO(H) blood group biosynthesis. *Nature Struct Biol*. 2002;9:9,685–690.
21. Owen 2008. Reid M, C Lomas-Francis. *The Blood Group Antigen Facts Book*. California: Academic Press; 1997. Schenkel-Brunner H. *Human Blood Groups: Chemical and Biochemical Basis of Antigen Specificity*. Wien: Springer-Verlag; 2000. Available via: http://www.owenfoundation.com/Health_Science/ABO_Blood_Cells.html.
22. Heinrich V, Ritchie K, Mohandas N, Evans E. Elastic thickness compressibilty of the red cell membrane. *Biophys J*. 2001;81:1452–1463.
23. Pauly H, Schwan, HP. Dielectric Properties and ion mobility in erythrocytes. *Biophys J*. 1966;6:621–639.
24. Chang H-C. Nanobead electrokinetics: The enabling microfluidic platform for rapid multi-target pathogen detection. *AIChE J*. 2007;53:10,2486–2492.
25. Vo-Dinh T. *Nanotechnology in Biology and Medicine*. Boca Raton, FL: CRC Press, Taylor and Francis Group; 2007(Chapters 5,10,12,16,17,19,22,28,31,33,36).
26. Minerick AR, Thibaudeau G. Detection Using Confocal Microscopy. In: Dongqing L, ed. *Encyclopedia of Micro- and Nanofluidics*. New York: Springer, Berlin Heidelberg; 2008.
27. Mannix RJ, Kumar S, Cassiola F, Montoya-Zavala M, Feinstein E, Prentiss M, Ingber DE. Nanomagnetic actuation of receptor-mediated signal transduction," *Nature Nanotechnol*, 2008;3:1,36–40.
28. El-Boubbou K, Gruden C, Huang X. Magnetic glyconanoparticles: A unique tool for rapid pathogen detection, decontamination, and strain differentiation. *J Am Chem Soc, Communication*. 2007;129(44);13392–13393.
29. Cross SE, Jin YS, Rao J, Gimzewski JK. Nanomechanical analysis of cells from cancer patients. *Nature Nanotechnol*. 2007;2:12,780–783.
30. Tysseling-Mattiace VM, Sahni V, Niece KL, Birch D, Czeisler C, Fehlings MG, Stupp SI, Kessler JA. Self-assembling nanofibers inhibit glial scar formation and promote axon elongation after spinal cord injury. *J Neurosci*. 2008;28:14,3814–3823.
31. Khang D, Park GE, Webster TJ. Enhanced chondrocyte densities on carbon nanotube composites: The combined role of nanosurface roughness and electrical stimulation. *J Biomed Mater Res A*. 2008;86A:1,253–260.
32. Shatkin JA. *Nanotechnology: Health and Environmental Risks*. Perspectives in Nanotechnology Series. Boca Raton, DL: CRC Press: Taylor & Francis Group; 2008.
33. Blaser SA, M Scheringer M, MacLeod M, Hungerbuhler K. Estimation of cumulative aquative exposure and risk due to silver: Contribution of nano-functionalized plastics and textiles. *Sci Total Environ*. 2008;390:396–409.
34. Benn TM, Westerhoff PK. Fate and transport of ionic and nanoparticle silver released from commercially available socks. The 235th ACS National Meeting, New Orleans, LA; April 6–10, 2008.
35. Abaxis®. *Piccolo Express Blood Chemistry Analyzer*. http://www.abaxis.com/index.asp. (Accessed June 2008).
36. Montagne F, Popa AM, Pugin R, Heinzelmann H. Stimuli-responsive polymers in bioMEMS devices. *NSTI-Nanotech*. 2008;2:653–656.
37. Pregibon DC, Toner M, Doyle PS. Multifunctional encoded particles for high-throughput biomolecule analysis. *Science*. 2007;315:5817,1393–1396.
38. Shaikh FA, Ugaz VM. Collection, focusing, and metering of DNA in microchannels using addressable electrode arrays for portable low-power bioanalysis. *Proc Nat Acad Sci USA*. 2006;103:13,4825–4830.
39. Tok JB-H, Chuang FYS, Kao MC, Rose KA, Pannu SS, Sha MY, Chakarova G, Penn SG, Dougherty GM. Metallic striped nanowires as multiplexed immunoassay platforms for pathogen detection. *Angew Chem Int Ed.* 2006;45:41,6900–6904.
40. Klibanov AM. Permanently microbicidal materials coatings. *J Mater Chem.* 2007;17,2479–2482.