Miniaturization Technologies for Molecular Diagnostics

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Background: Molecular diagnostics devices are becoming smaller. With the advancement of miniaturization technologies, microchip-based systems will soon be available for genetic testing. The purpose of this review is to highlight the underlying principles in miniaturization, the strategies being developed for bioanalysis, and the potential impact on the practice of this rapidly growing medical discipline.

Approach: The author discusses DNA microchips and their practical importation into the clinical laboratory, based on his background in medical device and microchip design and development. His discussion is supported by a body of literature covering both biomedical and electrical engineering and more recent publications in the field of molecular genetics and pathology.

Content: This review is descriptive and intended to outline the technologic and methodologic approaches to the creation of an integrated genetic analysis instrument based on miniature components. The review draws on published scientific evaluations of these devices without regard to the companies involved in their development.

Summary: The intent of this review is that the reader will better understand the variety of technical approaches toward the miniaturization of molecular genetic testing for the clinical laboratory. With insight into the principles underlying the operation of these chips and the integrated systems, the end user can better evaluate the value to the field in terms of making molecular genetics testing simpler, faster, and less expensive.

Two Revolutions

There are two revolutions underway in the clinical laboratory, and they are both likely to reshape the way we practice medicine. The first revolution is about how to organize, innovate, and implement new technologies to make laboratory testing less costly and less of a burden on the healthcare system. The second revolution is about finding intelligent ways to make sense out of, and then apply some of the information we get from genetic testing. For better or worse, molecular diagnostics, a discipline that uses DNA or RNA combined with molecular genetics methods to do diagnostic testing for patient care, is at the intersection of these two revolutions.

Over the past 10 years, the field of molecular diagnostics has experienced phenomenal growth and now represents a $1.2 billion industry. Recognizing this potential, in 1997, writers from Fortune magazine admonished investors to look beyond the hype of cloning “Dolly” to the riches to be gained by sequencing the human genome and to place their bets on the exciting new biochip industry. Actually, the potential of miniaturizing molecular diagnostics was foreseen by those who inspired the Human Genome Project and thus made one of its primary goals support of the invention of new technologies that make the process of gene discovery faster and less expensive. By and large this goal has been met: the cost of sequencing a DNA fragment is now 300 times lower than when the program started. In July 2000, the rough draft of the sequence of the human genome was completed, demonstrating not only the power of the science, but also the vitality of the private and public collaboration that made this feat possible. A similar partnership is fueling the biochip revolution, which will aid in proofreading of this initial draft as well as encouraging the development of useful applications of this information. At last count, a handful of large companies and >100 start-up companies were committed to the commercialization of miniaturized instruments for molecular diagnostics, including thermocyclers, DNA microarrays, and other types of biosensors.

Reality Check

As physicians become versed in the use of genetic information and gain an appreciation of how gene-based testing can be used to manage patients, the reliance on molecular diagnostics is expected to accelerate. However,
unlike other technologies currently used in clinical laboratories, molecular diagnostics is highly labor-intensive and lacks automation and integration of the various operational steps (1). With the impetus to offer a wider variety in molecular tests, the reality check for the laboratory director is how to address mounting clinical needs (market), but at the same time work to reduce costs. With the advent of miniaturization technologies, we are well on our way to seeing routine use of biochips as a solution to this dilemma.

For the most part, these technologies are still very much works in progress, and for this reason, the usefulness of these biochips is limited to applications in the research laboratory. Correspondingly, as companies have evaluated the contentious landscape for patent protection, the rigorous requirements of the Food and Drug Administration, the standards for quality control in clinical testing, and the diminishing opportunity for adequate test reimbursement in the molecular diagnostics market, many have shied away from biochips. Like any new generation of technologies, however, some biochip-based technologies show greater promise than others. In general, there are three questions to ask when assessing the potential of biochips for use in the clinical realm: Do they work? Do they help? Do they save time and/or money? This review provides some of the background necessary to answer these questions.

**Miniaturized Systems Really Means Only Chip-sized Biosensors**

Reduction in the costs of performing high-throughput DNA sequencing has been a major goal of the Human Genome Project. This goal inspired many of the “miniature systems” that now include such capabilities as DNA sequencing on a chip.

Correspondingly, reports that describe total-analysis microsystems, or TAS, for laboratory testing are usually referring to a combination of microchip-sized devices connected together or integrated on a single substrate (glass, plastic, or for select types of chip-based biosensor, silicon) used for the detection of nucleic acids or another type of analyte. Reducing costs is also a driving force in molecular diagnostics, and it is critical if this platform for testing is to compete with more conventional diagnostic methods. However, molecular diagnostics lack the extensive automation and system integration found in clinical chemistry laboratories (1). Hence, to appreciate where costs can be decreased in the operational schema of genetic testing requires an analysis of the whole system, including sample collection (Fig. 1). On the basis of current methods, sample collection and nucleic acid extraction are the most expensive steps involved in genetic testing (2). As a practical matter, however, the miniaturization of sample handling, involving principles of microfluidics and the controlled storage and delivery of biochemical solutions from reservoirs, has provided a more difficult set of engineering problems. Instead, research over the last 5–10 years has focused on adapting principles of miniaturization from the field of microelectronics to testing of new concepts for localizing biochemical interactions on chips the size of postage stamps. Industry leaders hope this strategy will afford the greatest likelihood of substantially decreasing costs (3).

In the effort to create a portable and inexpensive gene amplification and detection system, there has been the need to converge expertise in molecular biology and engineering. The goal remains to design miniaturized laboratory components that can be produced economically, are very small, and have performance superior to macroscale systems. Microelectromechanical systems
MEMS) are one potential solution because they leverage the economies of scale realized in the silicon processing industry while permitting the exploration of new principles of “sensing” not possible with conventional methods. The interface between these two disciplines has led to some exciting new ideas that only in the last few years have been realized as devices with potential commercial viability.

MEMS provide a means to make microminiaturized devices that do physical work such as pumping, valving, and oscillating; serve as actuators; and sense physical phenomena (sensors) by processes identical to those used to fabricate microelectronic chips. “Physical” sensors are the primary commercial application of MEMS, although new approaches toward combining the mechanical features of MEMS with biologic materials are laying the foundation for a whole new class of “biosensors”. MEMS sensors combined with microelectronic circuitry are sometimes referred to as “smart sensor systems”. Smart systems in turn can be configured into highly portable and inexpensive handheld or benchtop instrumentation. One potential advantage of MEMS devices is their cost of manufacture. Batch fabrication enables the production of thousands of devices at a cost that is only incrementally higher than the cost of manufacturing one. In some cases, MEMS-based gene chips are considered disposable, much like the plastics and biochemical reagents used in most laboratories.

The manufacture of MEMS involves processes common to the manufacture of microelectronic components, such as computer chips, from silicon, including photolithography, surface micromachining, and deposition of special materials such as zinc oxide, a piezoelectric composite. The microfabrication of a MEMS device is analogous to the construction of a house (Fig. 2). Before construction starts, blueprints of the house need to be drawn, creating a template designating the position of the walls and other key elements of that structure. Similarly, the process of photolithography leads to a series of photomasks, each outlining one layer of the three-dimensional MEMS structure. Each photomask is projected onto the surface of a silicon wafer, producing a pattern of shadows and illuminated areas. Photoresists are light-sensitive epoxies used in photolithography to differentially protect and deprotect areas of the wafer. Exposure to specific wavelengths of light causes these epoxies to cross-link or “cure”, or in the shadowed areas, become vulnerable to dissolution by organic solvents. Photolithography leaves a footprint for each detail of the structure on the wafer in a sequential placement of materials, much like a bricklayer might first build the foundation with bricks and cement in preparation for the footings of the vertical walls.

Another process in MEMS fabrication is the deposition of thin films. Typically, this involves the use of high temperatures and mixtures of gases, bathing the surface.

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Fig. 2. Schematic for the fabrication of a MEMS sensor.
Initial steps involve using photolithography to pattern areas where material is to be removed. (Step 1), a trench is created by etching silicon wafer with plasma, a reactive ion beam, or with chemical vapor. (Step 2), the trench is then filled with polysilicon glass (PSG) to protect that area from the subsequent processing, but which will be sacrificed at the last step. (Step 3), metals or special composite materials, such as thin films of piezoelectric ceramics, are then added to form the sensing elements. (Step 4), these materials are protected by a process of encapsulation with epoxies, called photoresists, or other thin films composed of nitrides or oxides of silicon. (Step 5), at the final step, the three-dimensional beam structure is formed through a process of chemical etching to remove the sacrificial materials. The result is a free-standing beam with electrodes and embedded piezoelectric actuators and sensors.
of the silicon wafer for precisely defined times and at precisely defined pressures. The result is a deposition of materials that have either electrical or structural properties useful to the finished device. Surface micromachining involves the use of a variety of techniques, including dry and/or wet etching procedures, to differentially remove materials from the silicon wafer and/or previously deposited thin films. Micromachining is performed in concert with serial photolithography steps to create structures with intricate details, including vertical walls, freestanding beams or diaphragms, conduits, valves, and specialized surfaces to affix biochemicals, such as nucleic acid probes.

The leveraging of MEMS and silicon processing technologies to create a genetic testing system on a chip is focused on two broad areas. The first is in the creation of miniaturized chambers, reservoirs, and conduits in which to carry out biochemical reactions such as PCR. The second area includes the creation of unique detection devices or sensors. Collectively, however, MEMS offer one of the biggest opportunities for the biotechnology industry.

**DNA Chip Technologies**

Anyone familiar with the method of using a slot blot, dot blot, or reverse blot to “probe” a sample, such as that derived from PCR, understands the principles of a DNA array. The placement of a series or array of oligonucleotide probes onto solid substrates such as nylon, paper, and polypropylene is an extension of using blotting methods to localize the signal when DNA fragments hybridize to their cognate complements. Various chemistries have been used to attach DNA probes to filters, but most involve a carboxyimide coupling using 5’-amino-modified nucleosides placed directly on the surface with a mechanical micropipet. Other methods depend on initially modifying the surface with a stable aliphatic poly linker followed by attachment using standard phosphoramidite chemistry (4). Microaddressable arrays or DNA chip arrays are a unique combination of technologies wherein microfabrication of silicon or glass is combined with novel ways of affixing gene probes to a silicon or glass surface. The origin of this technology is based on research by Foder and co-workers (5, 6), who used the technique of photolithography for in situ chemical synthesis of biochemicals directly on a silicon substrate. This so-called light-directed chemical synthesis incorporates nucleotides modified with photosensitive linkers as the building blocks in serial rounds of probe assembly (7).

The process begins when photosensitive linker molecules, which include amino acids or hydroxyl-photoprotected deoxynucleosides, are covalently attached to silicon wafers when pinpoint selected areas are illuminated through a photolithographic mask, which causes the linkers to be photodeprotected. In the next round of synthesis, the linker is reacted with the subsequent nucleotide in solution to form the oligonucleotide probe, but only at the positions exposed to light. The process is repeated, each time using a different photomask to expose a new geographic position on each of the hundreds of chips that are replicated across the wafer. After the first layer of nucleotides is fixed at each probe location, the process is repeated for the second row of nucleotides. The process permits incredible chemical diversity, where only 4 × N chemical steps can produce a complete set of 4N oligonucleotides of length N, or any subset on a single chip. Depending on the resolution of the photolithographic mask and instrumentation, probe densities as high as 10^8/cm^2 can be achieved, although densities 10 times lower are more typical (8). Current commercial versions of these chips hold up to 20 000–45 000 probes sites in a 1.28-cm^2 chip area. A recently reported variation of this approach uses precision direction control of a laser in lieu of photolithography to pinpoint the on-chip synthesis of oligonucleotide probes (9).

Affymetrix Corporation (Santa Clara, CA) uses this technology to make their Genechip™, which was the first DNA chip to be approved by the Food and Drug Administration for molecular diagnosis of reverse transcriptase gene mutations in HIV. Subsequently, several iterations of the Genechip have been configured for various research and clinical problems, such as mutational analysis of the p53 and BRCA1 genes and detection of single nucleotide polymorphisms in the cytochrome P450 system of enzymes. Importantly, the Genechip is only one component of a large benchtop system that includes a fluidic station, readout chamber, and a computer and software for interpretation of the data.

Directional placement of off-chip-synthesized oligonucleotide probes by electrostatic attraction is another method of constructing microaddressable array chips (10). Manufacture of the Nanogen chip involves fabrication of a dense array of tiny electrodes overlying a molecular permeability layer. A positive electrical bias applied to the electrode attracts negatively charged oligonucleotide probes to a precise location on the chip. Serial application of specific probes and differential activation of the electrode lead to the eventual assembly of a probe array (11).

Genometrix (Woodland, TX) uses off-chip-synthesized oligonucleotide probes but delivers them onto the chip by means of a microjet dispensing system (12). A variation of this strategy involves the deposition of polymers on the surface of the chip to embed generic oligonucleotides, called “zip codes”, at a precise location on the surface of a glass or silicon chip by means of a piezoelectric microdispenser (13). Through a process that involves the use of PCR combined with the ligase chain reaction or other gene amplification techniques, the products of amplification are then hybridized to the prefabricated gene chips, washed, and subjected to readout analysis (14, 15).
The readout of DNA chips involves detection of a reporter molecule, which typically is incorporated into the gene amplification product (Fig. 3). Incubation of the gene product, which is in solution, with the chip leads to hybridization of a segment of the amplicon sequence to the immobilized probe, ideally with a one-to-one complementarity. Specificity is achieved by optimizing the hybridization conditions, which depend mostly on the design of the probe. Interrogation of the hybridized chip by a laser to evoke fluorescence, or a chemical for chemiluminescence, is detected by a photomultiplier tube or a charge-coupled device, which ascribes the signal at a particular location on the array to the known sequence of the probe (16, 17). Knowing the pattern of product hybridization and the exact sequence of each probe, from the time when the chip was constructed, permits one to deduce the DNA sequence of the sample being tested (18, 19).

In practice, however, routine use of these chips can be difficult. Principally, the problems relate to achieving a reliable signal-to-noise relationship for each hybrid duplex sequence. This is more complicated when chips are used that involve the hybridization of products from a multiplex of amplification reactions containing numerous potential single-nucleotide differences. In fact, difficulties in interpreting fluorescence-based chips have led many investigators to use a manual readout scheme in which the chip is examined through a fluorescence microscope instead of with an automated chip reader and associated software system (20). However, turning to manual override is not possible with some of the recent chip constructs, where as many as 25,000–48,000 independent gene sequences are analyzed simultaneously.

At the molecular level, many chemical as well as physical factors influence the “quality” of the hybridization reaction, including steric hindrance of the fluorochrome, errors in or degradation of the probe sequence, and orientation of the probe against the surface of the chip (21). For these reasons, the development of zip codes has advantages in that each probe is designed to have closely matched physical characteristics and nearly uniform chemical properties, such as melting temperature. The result is markedly improved signal-to-noise discrimination and easy interpretation of the data. Moreover, Witowski et al. (22) have shown that by creating laminations of zip code probes embedded into acrylamide and acrylamide co-polymer, three-dimensional spots can be created that increase the probe density and hence the intensity of the fluorescence at a single point.
**Little Chips, Big Boxes**

In each of the above examples as well as in other versions of microaddressable array technologies, the reliance on an optical detection system to readout the DNA chip necessitates using large and expensive instrumentation. For this reason, the primary experience with this technology has been in large research facilities and for applications in drug discovery where high throughput and a high output of information are paramount (23). The successful application of DNA microarrays for gene discovery and cDNA expression arrays for disease profiling is in fact driving the direction of the technical refinement of these chips toward ones that contain more sequences and require more powerful and sophisticated analytic capabilities. These super chips are very expensive, and the big boxes that read them are increasingly out of the price range of most hospital-based laboratories, making the utility of these devices in the clinical arena and at the point of care seem more remote than when they were introduced.

A promising handheld DNA analyzer developed by Clinical Microsensors (Pasadena, CA) eliminates the need for an optical readout by sensing the change in electrical impedance when a DNA substrate is bound to a complementary probe compared with an unbound probe. In this case, the DNA probes are attached to electrode pads on the “chip” through molecular wires (made of a phenylacetylene polymer) (24). After hybridization of an unamplified DNA sample to the probe, DNA linked to a ferrocene redox label is added. When voltage is applied to that particular electrode, charge is conducted through the duplex DNA molecule and will have lower impedance, which is measured by the difference in voltage between bound and unbound probe electrodes (25).

**Microfabricated Genetic Devices**

One of the first demonstrations of a microfabrication technology for laboratory testing is a tiny microcapillary electrophoresis chip. A chip-based system is created using the technique of surface micromachining on glass, plastic, or silicon, etching a capillary up to 2 m in length in a space no larger than that of a postage stamp. At each end of the microcapillary is a fluid reservoir. Application of a voltage across these reservoirs will cause fluid to flow along the length of the microcapillary. Analytes, such as dissolved DNA fragments of various lengths, will separate according to their electrophoretic mobility countered by the oppositely directed electroosmotic flow. Additional reservoirs, connected by intersecting microcapillaries, permit directional flow of the solution and hence processing of specific analytes to their respective “chemical stations”. Caliper Technologies (Palo Alto, CA) incorporates this differential “electrokinetic flow” into their Lab on a Chip™ devices (26–28). A simpler version of a microcapillary electrophoresis system has been evaluated for several genetic markers, including bcr-abl gene transcripts associated with chronic myelogenous leukemia and separation of PCR fragments generated in the factor V Leiden mutation assay, as well as a multiplexed reaction involving mutational analysis of the cystic fibrosis gene (29–31). In each case, the clear advantage of microcapillary electrophoresis is its high throughput where the separation times are on the order of seconds from the time of sample application.

True MEMS sensors have only recently been shown to also function as biochips, although none have been demonstrated commercially for clinical applications. In a larger sense, MEMS devices take advantage of certain mechanical properties of structures that have homologs in the macroscale world. One such device is a so-called quartz microbalance, in which bulk monocrystalline quartz or a microfabricated cantilever-like structure is combined biochemically with certain probes of known mass (25, 32, 33). Quartz, having piezoelectric properties, can be put into a high-frequency oscillatory mode where the frequency of this oscillation is dependent on the mass of the structure. In this case, the DNA probe serves as a receptor that then hybridizes to its cognate DNA template, producing a structure with a mass larger than those of devices not containing bound DNA (34). The resulting device has a resonant frequency that is shifted downward or lower than those not displaying specific strand hybridization. A quartz microbalance array has been constructed and has been demonstrated to show high sensitivity of DNA hybridization to as little as $10^{-18}$ moles, or $\sim 10^{-12}$ g of target DNA (35).

Other researchers who constructed a surface acoustic wave sensor that uses thin-film piezoelectric materials exploited variations on this theme (36). In this case, DNA probes or other biomolecules immobilized on a planar surface are set into oscillatory mode by virtue of a microfabricated transmitter. On the opposite side is a microfabricated receiver. An oscillatory signal is broadcast across the piezoelectric field and is detected at the receiver at a known time, which is dependent on any impedance of that transmitted signal, such as that caused by bound DNA. Wang et al. (37) demonstrated the utility of the so-called flexural plate wave sensor for the detection of serum-based proteins through their affinity to a monoclonal antibody affixed to the piezoelectric sensor surface. McGlennen and co-workers have demonstrated how other types of piezoelectric thin films can be applied to structures such as microfabricated diaphragms and/or microcantilevers that detect mass changes with DNA hybridization (unpublished results; Fig. 4). In this case, the microcantilever is set into a fixed oscillatory mode that is altered when hybridization between PCR-derived DNA and its cognate oligonucleotide probe takes place (Fig. 5). These devices are sensitive to picograms of mass loading but demonstrate the added advantage of compatibility with on-chip microelectronic circuitry. One application demonstrating the potential for clinical use has been the detection of bacterial contaminants in milk (38).

Taken in total, these sensor systems can achieve high sensitivities not observed previously with addressable
array-based approaches and in turn can be linked directly to microelectronic circuitry, producing a device that is highly compact and very inexpensive to manufacture (39). One proposed device involves a MEMS microcantilever array for gene-based testing, illustrating how detection of mass loading by a shift in resonant frequency of several DNA products has the potential for enhanced diagnostic sensitivity. Moreover, the commercialization of MEMS-based sensors offers the promise of lower manufacturing costs, where individual devices can cost less than $1.00.

Surface plasmon resonance is yet another attractive technique to directly detect unamplified DNA samples. Surface plasmon resonance is based on intermolecular changes in the refractive index at the surface of the sensor over time (40). In this technique, a DNA template, unamplified or amplified, is hybridized in a small reservoir containing an optical interface bound to specific oligonucleotide DNA. Through the process of complementary-based hybridization, light passing through the sensor base will be refracted at an angle greater than in those sensors not demonstrating specific hybridization (41). This process can be calibrated by varying the temperature and is somewhat dependent on time. The specific conditions and kinetics of this interaction, in turn, are characteristic of the degree of sequence complementarity between the DNA template and the cognate probe. Work by Nilsson et al. (42) has demonstrated the utility of surface plasmon resonance in the detection of clinical samples amplified by PCR for a series of clinically relevant gene markers, including p53.

Changes in the optical pathlength have also been demonstrated to be an effective means of showing DNA hybridization on a silicon microchip interferometer (43). An interferometer is a device consisting of an array of micromachined pillars or finger-like projections that are created by etching deep into a silicon wafer. Incident light shown onto this area of micropillars will be reflected backward at a predictable angle of efferent light. When the effective thickness of an individual pillar is increased

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**Fig. 4.** Diagram of a microcantilever biosensor.

The MEMS component comprises a microcantilever beam constructed from silicon nitride. Overlying this basic beam is a series of layers that include a bottom electrode, an insulator, and a top electrode. In this example, a thin film of the piezoelectric composite material, lead zirconium titanate (PZT), is deposited, patterned, and bonded to the electrode stack. The piezoelectric film serves two functions. One set of piezoelectric strips actuates the beam to oscillate up and down when an AC voltage is applied. A second piezoelectric element senses that motion or any changes in the oscillatory frequency. Overlying the piezoelectric stack is the biomolecular recognition surface. This layer is composed of either a polymer or a self-assembling monolayer containing an embedded probe (an example using an oligonucleotide is described in the text). When a complementary sequence (such as that generated by PCR) is hybridized to the surface probes, the interposed polymer undergoes a conformational change and imparts a bending force to the beam. That force in combination with the added mass of the bound DNA is detected by the sensing piezoelectric element as a change in the resonant oscillatory frequency and is transduced into a measurable voltage.

**Fig. 5.** MEMS piezoelectric microcantilever sensor.

(A), photomicrograph of a piezoelectric microcantilever sensor. The dimensions of the device in this example are 100 μm x 75 μm, and the beam is 1.5 μm thick. The lateral strips are the actuator elements, and the center strip is the sensor element. (B), output of the oscillating biosensor. The resonance signals produced by the unbound (peak on the right) and bound (peak on the left) probes are shown.
by coating with DNA probe and subsequent hybridization of product to the DNA probe, there is a change in the effective pathlength of the light passing through the column, creating what are called Perot-Fabry fringes. The shift in the angle of the efferent light is proportional to the quantity of DNA hybridized to the affixed probe. Thus, the technology is highly specific because of the complementarity of DNA-DNA and may also show promise for quantitative analysis.

Each of the above approaches in some way involves exploitation of either physical properties inherent to DNA or the capability of light-based detection systems to resolve isolated areas of DNA hybridization. Another sensor system involves the combination of complex simulations of biological systems confined to a silicon microchip. These devices are generating great excitement because they appear to be a truly universal platform for biosensors of the future.

A biosensor that uses an artificial ion channel was reported by Cornell et al. (44). This device takes advantage of the sensitive discrimination of naturally occurring receptor systems and the resolution of natural and artificial biomembranes to function as a transducer and amplify signals in a highly compact and reproducible system. Briefly, a lipid bilayer is constructed that contains a known quantity of the artificial antibiotic gramicidin. Gramicidin exists in a homodimeric form wherein both monomers of the protein reside in a linear conformation. Intact homodimeric gramicidin permits the flow of cations across the lipid membrane through a central pore. To function as a biosensor, one subunit of gramicidin is covalently linked to a specific cellular receptor, such as thyroid-releasing hormone receptor, or a hapten directed against some analyte. Another hapten is tethered and directed to the lipid membrane. When a ligand, such as thyroid-releasing hormone, binds to its cognate receptor, there is competition between the tethered hapten and the one linked to gramicidin. The result is a disruption in the formation and continuity of the gramicidin transmembrane pores with a net loss of positively charged ions flowing across the membrane. This change in current can be detected by a galvanometer, which measures the absolute loss or decrease in current flow and additionally can record the phenomenon as a function of time.

The opportunity to use any of the biosensors listed above is indeed great. By design, many of these approaches can be adapted to a variety of test types, and each has the promise of high sensitivity and specificity (Table 1). The possibility for miniaturization is also apparent, but the commercial application of these for clinical gene-based diagnostics remains to be realized. The greatest challenges, however, lie in the integration of sensing technologies with those “front-end events” such as sample preparation and, in the case of PCR, thermocycler-based assays. Companies such as Cepheid have focused on technologies to miniaturize blood separation and DNA purification using chips by fabricating a mechanical sieve

<table>
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<tr>
<th>Type of biochip</th>
<th>Technical principle</th>
<th>Development status</th>
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<tr>
<td>Microcapillary electrophoresis</td>
<td>Separation of analytes by electrophoresis in a microfabricated capillary 1–30 µm in diameter</td>
<td>Commercialized by Cepheid and Caliper Technologies, and Soane Technologies</td>
</tr>
<tr>
<td>DNA hybridization chip</td>
<td>Synthesis or site-directed fixation of oligonucleotide probes onto chip surface; detection by laser-induced fluorescence of hybridized PCR product</td>
<td>Commercialized by Affymetrix for several diagnostic tests including HIV, p53, and BRCA1; other manufacturers are involved in research markets or awaiting FDA approval</td>
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<tr>
<td>Surface acoustic wave sensor (SAW)</td>
<td>Detection of change in the propagation of a surface wave from transmitter to receiver through a molecular binding domain</td>
<td>Research device developed at the University of California, Berkeley</td>
</tr>
<tr>
<td>Surface plasmon resonance (SPR)</td>
<td>Detection of the evanescent wave propagated from the interface of a reflecting metal to which is bound a hybridized analyte</td>
<td>Benchtop instrument commercialized by Biacore; microchip-based version developed by University of Minnesota</td>
</tr>
<tr>
<td>Electromechanical MEMS sensor</td>
<td>Detection of mass loading, either by shift in resonant frequency or by static flex of a piezoelectric transducer</td>
<td>Bioanalytic Microsystems, University of Minnesota</td>
</tr>
<tr>
<td>Porous silicon interferometer</td>
<td>Silicon is “roughened” to increase the surface area; oligonucleotide probes are fixed to the surface; light reflected from the surface will shift in wavelength as a function of the change in the optical thickness attributable to sample binding</td>
<td>Research development at several centers</td>
</tr>
<tr>
<td>Artificial biomembrane sensor</td>
<td>A phospholipid bilayer containing a synthetic protein, e.g., gramicidin, over a gold electrode creates ionic pores that permit a current to cross the membrane; distortion of the pores because of binding of a ligand to a “tethered” receptor decreases the ionic current, which is measured by a galvanometer</td>
<td>Developed commercially by Dunlop Corporation</td>
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* FDA, Food and Drug Administration.
and combining this extractor chip with a chip-based reaction chamber for thermocycling.

Integration Is the Key

Reduction of cost is the major concern in laboratory medicine, and nowhere is that truer than in molecular diagnostics. To appreciate how chip technology and miniaturization in general can impact the cost of molecular diagnostics requires a thorough examination of each of the steps involved in performing that test. On the basis of currently available technology, molecular testing is at best a collection of piecemeal operational steps, each of which is largely manual, and requires expert personnel to perform tests accurately and reliably. Currently, the “hows” and “whys” of specimen collection, DNA extraction, various methods for nucleic acid amplification, and the choice of analytic techniques for the products of those amplifications are each being worked on as individual pieces of technology, some of them chip-based and others not. Unlike disciplines such as clinical chemistry, where highly refined and automated instrumentation is commonplace, molecular diagnostics methods boast little or no automation or system integration. Luckily, molecular diagnostics will be the first to benefit from recent discoveries in biochip technology and the convergence of the seemingly unrelated fields of electrical and mechanical engineering and material science with the breakthrough in commercialization of molecular biology reagents. Innovators in the biotechnology industry are realizing that the path taken to the mass production of computer chips may also lead to miniaturized laboratory chips, with the commensurate performance, integration, and cost savings needed for gene testing to fulfill its potential in clinical practice. So promising are the prospects for chip technology in the diagnostic laboratory that one outcome will be realization of instruments for point-of-care or, possibly, at-home gene testing.

Conclusion

As is often the case with breakthrough technologies, there is a great deal of drama surrounding the prospect of creating micrometer- and even nanometer-scale chips, known as biochips. However, in this case, much of this optimism is justified, considering that in just a few short years basic research has led to several products that are in or near the commercial phase of their development. Developers of DNA chips believe that in the near future these technologies will enable clinicians, and in some cases patients themselves, to quickly and inexpensively detect a wide variety of genetic-based diseases and conditions, including AIDS, Alzheimer disease, cystic fibrosis, and several forms of cancer. In other arenas, this technology will also make it possible to develop inexpensive strategies for screening of new pharmaceutical agents as well as new genes associated with hitherto uncharacterized diseases. Until then, however, considerable work needs to be done to make these technologies useful and robust as well as inexpensive for the end user. To date, a few companies have commercialized DNA chip products, but many more underlying scientific principles remain to be discovered before these devices are ready for routine clinical use.

The author is president of Access Genetics, Inc.

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