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Research review paper

Biosensor technology: Technology push versus market pull

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ABSTRACT

Biosensor technology is based on a specific biological recognition element in combination with a transducer for signal processing. Since its inception, biosensors have been expected to play a significant analytical role in medicine, agriculture, food safety, homeland security, environmental and industrial monitoring. However, the commercialization of biosensor technology has significantly lagged behind the research output as reflected by a plethora of publications and patenting activities. The rationale behind the slow and limited technology transfer could be attributed to cost considerations and some key technical barriers. Analytical chemistry has changed considerably, driven by automation, miniaturization, and system integration with high throughput for multiple tasks. Such requirements pose a great challenge in biosensor technology which is often designed to detect one single or a few target analytes. Successful biosensors must be versatile to support interchangeable biorecognition elements, and in addition miniaturization must be feasible to allow automation for parallel sensing with ease of operation at a competitive cost. A significant upfront investment in research and development is a prerequisite in the commercialization of biosensors. The progress in such endeavors is incremental with limited success, thus, the market entry for a new venture is very difficult unless a niche product can be developed with a considerable market volume.

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1. Introduction

The field of biosensor technology was originated from the papers by Clark and Lyons (1962), Guilbault et al. (1962), Updike and Hicks (1967) and Guilbault and Montalvo (1969). Di Gleria et al. (1986) described a mediated electrochemical biosensor using ferrocene instead of dioxygen to alleviate electroactive interfering species such as uric and ascorbic acids. This elegant procedure formed the basis for successful commercialization of a glucose pen by Medisense. A biosensor is defined by The National Research Council (part of the U.S. National Academy of Sciences) as a detection device that incorporates a) a living organism or product derived from living systems (e.g., an enzyme or an antibody) and b) a transducer to provide an indication, signal, or other form of recognition of the presence of a specific substance in the environment. As a self-contained integrated receptor-transducer device, a biosensor consists of a biological recognition element in intimate contact or integrated with a transducer. Ideally, biosensors must be designed to detect molecules of analytical significance, pathogens, and toxic compounds to provide rapid, accurate, and reliable information about the analyte of interrogation. Biosensors have been envisioned to play a significant analytical role in medicine, agriculture, food safety, homeland security, bioprocessing, environmental and industrial monitoring. After the September 11, 2001 event, the detection of biohazards in the environment has become an important issue (Fuji-Keizai USA, Inc., 2004; Rodriguez-Mozaz et al., 2005) as reflected by a significant increase in funding for biosensor research in relation to homeland security in the USA and some other countries (Fuji-Keizai USA, Inc., 2004) towards the development of hand-held biosensor technology. Recent incidences of contaminated foodstuffs have also heightened consumer concern. Lab tests for bacterial contamination in meat are required by regulators, but they are costly and slow; only yielding results after 2 to 3 days. Hence, food products remain stored in warehouses for longer periods. Albeit a plethora of workable biosensors for a variety of applications has been developed, besides the blood glucose and lactate biosensors and a few other commercial hand-held immunosensors in clinical diagnostics, only a minimal number of biosensors appear to be commercially feasible in the near future.

Annual worldwide investment in biosensor R&D is estimated to be $300 US million (Weetall, 1999; Alocilja and Radke, 2003; Spichiger-Keller, 1998). Both publications and patents issued are phenomenal in biosensor research. From 1984 to1990, there were about 3000 scientific publications and 200 patents on biosensors (Collings and Caruso 1997; Fuji-Keizai USA, Inc., 2004). The same number of publications (~3300 articles) but almost double the patent activity (400 patents) was noticed from 1991 to 1997. The explosion of nanobiotechnology from 1998 to 2004 had generated over 6000 articles and 1100 patents issued/pending (Fuji-Keizai USA, Inc., 2004). Thus, significant improvements in the biosensor performance in terms of selectivity and detection sensitivity, at least under well-controlled environments, have been realized to facilitate the applications of various biosensors. Such impressive publications and patents, doubtlessly, suggest a continuing bright future for R&D activities in biosensor technology with the health, drug discovery, food, homeland security, pharmaceutical and environmental sectors as the major beneficiaries (Hall, 1990; Andreescu and Sadik, 2004; Turner, 1996). However, the commercialization of biosensor technology has significantly lagged behind the research output. The rationale behind the slow technology transfer could be attributed to cost considerations and some key technical barriers such as stability, detection sensitivity, and reliability. The laboratory diagnostics market has changed considerably in the last decade and innovation in this segment will be increasingly driven by automation and system integration with high throughput for multiple tasks. Such requirements pose a great challenge in biosensor technology which is often designed to detect one single or a few target analytes. In addition, before the biosensor gains market acceptance, it must prove its effectiveness in the field test followed by its validation by well-established procedures. Lab studies with “fairly clean” samples often fail to provide an adequate measure of capability for “real-world” samples, leading to failed technology transfer and further investment. Such activities require appropriate sources of finance for technology development and demonstration. Ultimately, the success of biosensors must prove that it is the inevitable choice as a cost-effective analytical tool.

This report aims to provide an overview of biosensor technology with some highlighted advances in both the transducer element and the biorecognition molecule. Technical hurdles associated with the biosensor development/application in clinical chemistry, food safety, environment, and homeland security are addressed together with the identification of market opportunities and commercialization activities. These hurdles include relatively high development costs for single analyte systems and limited shelf and operational lifetimes of biorecognition components.

2. An overview at biorecognition elements and transduction technology

2.1. Transduction technology

Although a variety of transducer methods have been feasible toward the development of biosensor technology, the most common methods are electrochemical and optical followed by piezoelectric (Hall, 1990; Buerk, 1993; Wang, 2000; Collings and Caruso 1997). Electrochemical sensors measure the electrochemical changes that occur when chemicals interact with a sensing surface of the detecting electrode. The electrical changes can be based on a change in the measured voltage between the electrodes (potentiometric), a change in the measured current at a given applied voltage (amperometric), or a change in the ability of the sensing material to transport charge (conductometric). Electrochemical biosensors appear more suited for field monitoring applications (e.g. hand-held) and miniaturization towards the fabrication of an implantable biosensor. Based on their high sensitivity, simplicity and cost competitiveness, more than half of the biosensors reported in the literature are based on electrochemical transducers (Meadows, 1996). Optical sensors employ optical fibers or planar waveguides to direct light to the sensing film. Evanescent waves propagating from waveguides can be used to probe only the sensing film to decrease the optical background signal from the sample. The measured optical signals often include absorbance, fluorescence, chemiluminescence, surface plasmon resonance (to probe refractive index), or changes in light reflectivity. Optical biosensors are preferable for screening a large number of samples simultaneously; however, they cannot be easily miniaturized for insertion into the bloodstream. Most optical methods of transduction still require a spectrophotometer to detect any changes in signal. Mass sensors can produce a signal based on the mass of chemicals that interact with the sensing film. Acoustic wave devices, made of piezoelectric materials, are the most common sensors, which bend

when a voltage is applied to the crystal. Acoustic wave sensors are operated by applying an oscillating voltage at the resonant frequency of the crystal, and measuring the change in resonant frequency when the target analyte interacts with the sensing surface. Similarly to optical detection, piezoelectric detection requires large sophisticated instruments to monitor the signal. Nevertheless, the development of new and improved optical methods has the potential to replace electrochemical methods for the *in vivo* monitoring of pH, oxygen, and carbon dioxide concentration (Spichiger-Keller, 1998).

2.2. Biorecognition elements

Enzyme-based biosensors have been popular with over 2000 articles published in the literature and this is plausibly due to the need for monitoring glucose in blood (Tothill, 2001; D'Orazio, 2003) and the ease of construction of such biosensors. The use of enzymes as the biological recognition element was very popular in the first generation of biosensor development due to their commercial availability or ease of isolation and purification from different sources. Among various oxidoreductases, glucose oxidase, horseradish peroxidase, and alkaline phosphatase have been employed in most biosensor studies (Wang, 2000; Rogers and Mascini, 1998; Laschi et al., 2000). In most applications, the detection limit is satisfactory or exceeded but the enzyme stability is still problematic and the ability to maintain enzyme activity for a long period of time still remains a formidable task (Buerk, 1993; Tothill, 2001; D'Orazio, 2003). In some cases, electroactive interferences caused by endogenous compounds in the assay samples become significant and need to be suppressed. To date, glucose oxidase is still the most stable and specific enzyme which can be easily obtained in high quantity. Enzymes can be used in combination for detection of a target analyte, e.g., glutaminase together with glutamate oxidase for detection of glutamine (Male et al., 1993). The use of enzyme amplification to increase detection sensitivity is another important issue. For instance, glucose oxidase can be combined with glucose dehydrogenase to significantly improve the response signal (Gooding et al., 2000).

Since the last 15 years, affinity biosensors have received considerable attention since they provide information about binding of antibodies to antigens, cell receptors to their ligands, DNA/RNA to complementary sequences of nucleic acids and functioning enzymatic pathways (screening gene products for metabolic functions). The development of nucleic acid biosensors alone has resulted in over 700 papers published since 1997. The preferred methods of measurement include optical (SPR, Surface Plasmon Resonance), electrochemical or piezoelectric detection systems. The detection of specific DNA sequences has been advocated for detecting microbial and viral pathogens (Yang et al., 1997) as viruses are almost uniquely DNA or RNA composed within an outer coat or capsid of protein (Hall, 1990). In general, the DNA biosensor employs relatively short synthetic oligodeoxynucleotides for detecting target DNAs with the same length (Palecek, 2002). The system can be used for repeated analysis since the nucleic acid ligands can be denatured to reverse binding and then regenerated (Ivnitski et al., 1999). The peptide nucleic acid, an artificial oligo-amide capable of binding very strongly to complementary oligonucleotide sequences has been attempted (Vo-Dinh and Cullum, 2000). The electrochemical platform is popular since it is ideal for studying DNA damage and interactions (Fojta, 2002). However, considerable research is still needed to develop methods for directly targeting natural DNA present in organisms and in human blood (Palecek, 2002) with high detection sensitivity. Significant attention has also focused on improving the detection methods for DNA hybridization (Palecek, 2002). The hybridization event has been detected via electroactive or redox indicators such as metal coordination complexes or intercalating organic compounds (Peng et al., 2002; Wong et al., 2004; Meric et al., 2002; Ju et al., 2003; Babkina et al., 2004). Besides electrochemical detection, SPR has gained significant popularity in DNA sensing and other bioapplications. Measurements can be obtained directly, in minutes, rather than the hours required to visualize results of an ELISA (Spangler et al., 2001).

Based on the high selectivity of the antibody–antigen reaction, the development of hand-held immunosensors for infectious diseases has received considerable attention, driven mainly by the need for point of care measurements, homeland security and environmental monitoring. Analytes containing a mixture of protein can also be immobilized onto an antibody-coated surface of support in an array format (Huang et al., 2004). The presence of protein in analytes is detected with biotin-labeled antibody coupled with an enhanced chemiluminescence or fluorescence detection system. The exact amount of protein can be quantitatively measured. There are at least 800 papers reported in the literature on immunosensors and a more detailed description of immunosensors is available from the literature (Stefan et al., 2000). Antibodies are the critical part of an immunosensor to provide sensitivity and specificity. As the antibody–antigen complex is almost irreversible, only a single immunoassay can be performed (Buerk, 1993) although intensive research effort has been directed toward the regeneration of renewable antibody surfaces. Reproducibility is another concern, partly due to unresolved fundamental questions relating to antibody orientation and immobilization onto the sensor surface. Thus, immobilization of a receptor to the sensor surface is of central importance to the design of a successful biosensor assay. Affinity-capture and sulfydryl couplings can be used to produce a more homogeneous population of oriented receptors on the surface (Catimel et al., 1997). Last, immunosensors have to compete with well-established immunoassays which have become a standard tool in clinical and hospital settings using highly automated instruments used to analyze a number of samples in a short time frame (Hennion and Barcelo, 1998).

3. Technical hurdles and market potentials

Marketable viability will depend on whether a biosensor is versatile and inexpensive for a wide range of applications. Many technical issues remain problematic regardless of the type of biosensor platform. First, the commercially viable biosensor must function continuously over a long period with a lifetime of at least 1 month. Besides the glucose meter, most of the biosensors cannot fulfill this stringent requirement due to the fragility of the biorecognition element. Second, only a few biosensors can accurately assay a biological sample in less than a few minutes while most devices have an analysis time ranging from 15 min to several hours. Problems associated with matrix interference, sensor fouling due to adsorption of endogenous components in the assay sample, signal drift, and microbial contamination are common for all biosensors. Many of the biosensor innovations have performed well under controlled environments and have been only subjected to limited evaluation using pristine laboratory samples. Last, significant activities are needed to compare the biosensor's performance with established protocols to get the approval from regulatory agencies if the product is intended for medical applications. The financial and technological risks associated with this step can be very high and unpredictable. Other obstacles include a limited market for analysis of individual compounds or compound classes. Hence, successful biosensors must be versatile enough to support interchangeable biorecognition elements, miniaturization to allow automation and ease of operation at a competitive cost. Other desired features include automated, continuous and remote detection of multiple, complex analytes. Therefore, considerable technical challenges need to be overcome to tightly integrate biosensing platforms with sampling, fluidic handling, separation, and other detection principles.

The world biosensor market was $7.3 (US) billion in 2003 and was expected to reach over $10 billion by 2007 (Fuji-Keizai USA, Inc., 2004) with the medical/health area being the largest sector (Alocilja and

Radke, 2003). Similarly, another independent market report indicates that the global market for biosensors and other bioelectronics will grow from 6.1 billion in 2004 to 8.2 billion in 2009 (http://www.biz-lib.com/products/ZBU80661.html). Biosensors, particularly glucose sensors, accounted for nearly all of the market in 2003. The total worldwide medical biosensor sales was $7 billion (US) in 2004 and projected to be $8.3 billion (US) by the end of 2007 (Hall, 1990; Fuji-Keizai USA, Inc., 2004) with over 50% and 22% of the biosensor sales in North America and Europe alone. As expected, the glucose biosensor was the most widely commercialized of all biosensors (Newmann et al., 2002; Alocilja and Radke, 2003) considering the number of diabetic patients was 150 million in 2004. Although the number of diabetic cases could double to 300 million by 2025 (Newmann et al., 2004), the market of glucose biosensors is somewhat stagnant. The worldwide market for *in vitro* diagnostics was estimated to be about $17 billion in 2003 (Weetall, 1999) with molecular diagnostics as a fast growing area. Although the molecular diagnostics market was about $1.3 billion in 2003, it might reach $7 billion by 2010 (http://www.geneohm.com). The pharmaceutical research industry has a real need for biosensors to accelerate the progress of drug discovery and screening (Legge, 2004). The pharmaceutical industry with total worldwide biosensor sales in 2004 of about $577 million (US) was expected to grow to $1.5 billion (US) by the end of 2007 with over 50% sales in the North America biosensor market.

Public safety and concern, new legislation and recent food contamination in several countries have fostered a major research effort in the environmental and food/agricultural industry. There is an urgent need to ensure that food production and quality meet regulations (Fuji-Keizai USA, Inc., 2004). About 5000 people die each year from *Salmonella* and/or *E. coli* induced food poisoning in the USA (Fuji-Keizai USA, Inc., 2004). The global cost of the SARS outbreak was estimated to be 10–100 billion dollars while an outbreak of foot and mouth disease in the UK (2001) was about 5.8 billion dollars in reduced livestock production earnings. Consequently, the environmental and food industries are potentially emerging markets. The worldwide food production industry is worth about $578 US billion and the demand for biosensors to detect pathogens and pollutants in foodstuffs is expected to grow in the near future (Alocilja and Radke, 2003). The total market potential for detection of pathogens in the USA is about $563 million/year with an annual growth rate of 4.5% (Alocilja and Radke, 2003) compared to $150 million/year for the USA food industry sector. Considerable amount of work has focused on the development of biosensors to rapidly detect biowarfare agents. However, besides the USA and a very few countries, the biosensor market in the biosecurity/military industries in the near future is uncertain. A key issue for homeland security is absolute reliability as 'false negatives' are unacceptable. Too many 'false positives' cause stress and inefficiency, and quickly cause people to ignore warnings. Advances in areas such as toxicity, bioavailability, and multi-pollutant-screening, will widen the potential market and allow biosensors to be more competitive with conventional lab-based procedures.

4. Commercialization activities

About 200 companies worldwide were working in the area of biosensors and bioelectronics at the turn of the century (Weetall, 1999). Some of these companies are still involved in biosensor fabrication/marketing whereas others just provide the pertinent materials and instruments for biosensor fabrication. Most of these companies are working on existing biosensor technologies (Weetall, 1999) and only a few of them are developing new technologies. While the commercial market for blood glucose monitoring continues being the major driving force (over 85%), the commercialization of a hand-held biosensor for infectious disease detection can be projected within the next decade. Medical applications overshadow the other application sectors and could be attributed to the increasing rate of obesity and the alarming rise in the rate of diabetes in the industrialized countries. The SPR technology will gain significant attention and with miniaturization and cost reduction, SPR microarray will be a serious contender and competes head-to-head with electrochemical detection in both research and application.

One might pose a question: is the Biacore system a biosensor or just a lab-based system like HPLC, MS, etc? The classification of a biosensor becomes more intriguing and debatable due to significant advances in microfrabrication and nanotechnology. In the 1960s and 1970s, a biosensor was just a probe, somewhat similar to pH, ion selective or oxygen electrodes equipped with a simple readout device. As the sensing tip has been shrinking to micron and nanosize, other analytical instruments have also become smaller and smaller or even portable and are equipped with more robust and powerful data acquisition and processing. For instance, the room sized mass spectrometers of 1950 can be reduced to a few cubic centimeters. Miniaturized mass spectrometry, chromatography or electrophoresis chips have become feasible and might serve as a viable sensor component. In view of this, the definition of biosensor technology should be revisited to accommodate biosensors as a part of automated instruments. A typical example is the use of an AFM tip to form an AFM-based biosensor (Kaur et al., 2004). Of course, AFM-based biosensors have been developed by several other researchers; however, this paper is cited here because it was published in Biosensors and Bioelectronics, a journal which is dedicated to biosensor technology. Because of the comparatively large number of small and big companies that have engaged in some sort of commercialization, this review will not be able to cover all commercial activities in this field. The authors therefore apologize in advance to anybody or companies who feel that their activities in this field have been left out. Chromatography chips, microfabricated chips and hyphenated systems including microdialysis probes coupled to a detection system cannot be discussed here because of space limitations. Except for SPR technology, piezoelectric and other optical detection is not included due to its low market volume and or visibility.

4.1. Yellowsprings instruments (YSI)

In 1975, YSI (http://www.ysilifesciences.com) commercialized the first analyzer to measure glucose in whole blood. YSI followed this in 1982 with a whole-blood lactate analyzer. Since then, these products have become a standard for clinical diagnostic work at many sites in hospitals. The technology developed by Clark and Lyons over 45 years (Clark and Lyons, 1962) ago still provides fast, accurate glucose and lactate results in whole blood, plasma, serum, and cerebrospinal fluid. Up to 90 g/L glucose and 30 mmol/L lactate can be measured without the need for sample dilution and the results can be obtained in minutes. The analyzer's hematocrit correction option provides accurate glucose results expressed as plasma even when running whole blood. The analyzer requires only a small sample (25 μL), making it practical in neonate applications.

4.2. Nova biomedical

Nova's StatStrip™ Glucose Monitor (http://www.novabiomedical.com) has received clearance from the U.S. Food and Drug Administration for use in neonatal testing. Severe hematocrit abnormalities are routinely found in neonates and interfere with glucose measurement. StatStrip is the only glucose monitor with 6s analysis time that measures hematocrit on the strip, automatically correcting glucose values for abnormal hematocrit values. StatStrip measures and corrects electroactive interferences from acetaminophen, uric acid, ascorbic acid, maltose, galactose, xylose, and lactose. StatStrip also eliminates oxygen interference to provide accurate glucose results

regardless of the sample's oxygen level. The company also provides a hand-held device for the measurement of blood lactate (muscle performance indicator) using a very small drop of blood (0.7 µL) with an analysis time of 13 s. Nova also commercializes a biosensor that measures creatinine with an analysis time of 30 s and a wide range of BioProfile Analyzers for bioprocessing for monitoring glucose, glutamate, glutamine, glycerol, lactate, and acetate in addition to pH, pO_2, pCO_2, ammonium, and phosphate.

4.3. Abbott laboratories

Abbott Laboratories (http://www.abbottdiagnostics.com) acquired MediSense in 1996 for $867 million for the blood electrochemical glucose meter. Abbott then acquired TheraSense (blood glucose monitoring) and i-STAT for $392 million in early 2004, the latter being a company that commercialized a portable, hand-held analyzer for urea and blood gas analysis. In 2001, the company launched the Precision Xtra, the first personal blood glucose monitor with ketone testing capability. On Jan.18, 2007, Abbott sold its core laboratory diagnostics business included in the Abbott Diagnostics Division and Abbott Point of Care (formerly known as i-STAT) to GE for $8.13 billion. However, Abbott's Molecular Diagnostics and Diabetes Care (glucose monitoring) businesses are not part of the transaction and will remain part of Abbott.

4.4. Bayer AG (diagnostics division)

The company offers a variety of Glucometer® instruments for blood glucose testing and an *in vitro* diagnostic immunoassay system for hepatitis A virus. The company has received several granted patents, notably US Patent 6,531,040 that describes an electrochemical sensor for detecting analyte concentration in blood (http://www.bayerdiag.com). The Glucometer Elite® Diabetes Care System is a blood glucose monitoring system based on an electrode sensor technology. Capillary action at the end of the test strip draws a small amount of blood into the detection chamber and the result is displayed in 30 s.

4.5. Roche diagnostics AG

Roche Diagnostics (http://www.roche-diagnostics.com) biosensors permit near-painless, continuous measurement of blood glucose level. It markets the Accu-Chek family of products/services for blood glucose monitoring. Its US Patent Number 6,541,216 describes an invention that allows the measurement of blood ketone levels. The Accu-Chek Plus Glucose Meter is preloaded with a drum of 17 diabetes test strips, i.e., no individual strip handling with the test result appearing in 5 s.

4.6. Affymetrix

The Affymetrix (http://www.affymetrix.com/index.affx) GeneChip microarray is a workhorse in research institutes as well as pharmaceutical, biotechnology, agrochemical, and diagnostic settings. GeneChip microarrays consist of small DNA fragments or probes which are chemically synthesized at specific locations on a coated quartz surface. The precise location where each probe is synthesized is known as a feature, and millions of features are contained on each array. Nucleic acids extracted and labeled from samples are then hybridized to the array, and the amount of label can be monitored at each feature, resulting in a wide range of possible applications on a whole-genome scale, including gene- and exon-level expression analysis, novel transcript discovery, genotyping, and re-sequencing. Over 13,000 scientific publications have used this GeneChip technology. The company also has an impressive number of US patents issued and pending (230 and 420, http://www.affymetrix.com).

4.7. Biacore international AB (GE health care)

Surface plasmon resonance (SPR) biosensors are optical sensors exploiting special electromagnetic waves, surface plasmon-polaritons, to probe interactions between an analyte in solution and its corresponding recognition element immobilized on the SPR sensor surface. Based on SPR, Biacore's technology provides a non-invasive, label free system for studying biomolecular interactions. The company focuses on drug discovery and development (http://www.BIAcore.com) although it also provides a range of products for determination of food quality and safety. The first system was commercialized in 1989 followed by the second generation model (BiaCore 3000) with high performance in 2003, a system that has been well received in proteomic and clinical research (http://www.biacore.com/lifesciences/index.html). GE Health purchased Biacore, the largest SPR instrumentation, with 2005 sales of 76.8 million (http://www.allbusiness.com/instrument-business-outlook/1186240-1.html). Biacore is a multi-application research tool, offering a range of data output from yes–no binding data and concentration analysis to detailed affinity, specificity and kinetic data. This model also offers increased integration with mass spectrometry. There are over 2800 references citing Biacore across therapeutic areas including cancer, neuroscience, immunology and infectious disease.

It is of interest to note that in most Biacore applications, the ligands are tethered to a carboxylated dextran matrix that coats the chip surface. The carboxyl groups are capable of concentrating proteins at the surface and speeding up the immobilization process. Without this pre-concentration effect, ligand immobilizations can only be realized at concentrations above >1 mg/ml to drive the chemistry. In addition to its high cost (high-end instruments, $250,000–$500,000), BiaCore requires high-quality reagents with high activity, high non-specific binding, high stability, and/or high solubility. SPR array platforms also present a new level of technical challenges, including how to immobilize ligands and/or process large data sets efficiently. Presently, SPR biosensors can monitor up to 100 biological evaluations/day. The SPR array chip technology is expected to process 100,000 biological evaluations/day. Despite its versatility, the SPR system becomes less applicable for detecting biomolecules which have a molecular weight of less than 5000 Da. However, a surface-competition assay format was developed that allowed indirect detection of small-molecule binding (Zhu et al., 2000). Other improvement in SPR instrumentation has enabled detection of small molecules, such as drugs (≥138 Da) binding to human serum albumin (Frostell-Karlsson et al., 2000) and small oligosaccharides (<1000 Da) binding to an antibody (Hsieh et al., 2004). The long-term stability of the surface layer is questionable when in direct contact with blood and the signal is very sensitive to non-specific binding for real-time measurement in blood (Meadows, 1996).

4.8. Applied biosystems and HTS biosystems

Applied Biosystems (http://www.appliedbiosystems.com) and HTS Biosystems (http://www.htsbiosystems.com) jointly develop the 8500 Affinity Chip Analyzer. The technology is based on grating-coupled SPR and employs a single large flow cell so that 400 ligands can be spotted and analyzed at one time. This system is particularly well suited to examine antibody–antigen interactions and it can detect analytes with molecular masses down to 5000 Da (Applied Biosystems Application Notes about antibody characterization at http://www.appliedbiosystems.com/). For antibody, peptide, and DNA, the preparation of pertinent chips is relatively straightforward because these ligands retain their native structure throughout the preparation process involving drying and reconstitution steps. Patterning methods for more labile enzymes and receptors are still a formidable task and require more elaborate procedures. Nevertheless, the 8500 Affinity Chip Analyzer is expected to open up new possibilities for biosensor analysis.

4.9. BIND™ biosensor

In parallel processing, the delivery of separate samples to the detector in a rapid manner and at constant concentration is not an easy task. Although several microfluidics platforms have been developed to solve this problem, the SRU Biosystems (http://www.srubiosystems.com) uses special 96- or 384-well plates with a colorimetric resonant grating on the bottom. The system employs a guided mode resonant filter to monitor refractive index changes at the sensor surface. This label free system is designed for end-point measurements to tracks analyte binding in each well and the entire plate can be read within fifteen s. This standard microtiter plate format can be easily integrated with other robotic systems for sampling and data output.

4.10. LifeScan

LifeScan (http://www.lifescan.com), a part of the Johnson & Johnson companies, launched a painless stress-free glucose measuring device (OneTouch® Ultra® blood glucose) and the InDuo® system, the world's first blood glucose monitoring and insulin-dosing system, in 2001. In 2003, LifeScan launched the OneTouch® UltraSmart® blood glucose monitoring system with a 3000-record memory for the storage of health, exercise, medication, and meal information. The system combines an Ultra Soft™ Adjustable Blood Sampler for different puncture depths with One Touch® Ultra Soft Lancets for a less painful stick. The test requires a very small blood drop (1 μL) taken from either the finger or forearm, which is placed on a disposable test strip and the results are obtained in 5 s. LifeScan has an exclusive U.S. agreement with Medtronic to develop a new blood glucose meter that will wirelessly transmit glucose values to Medtronic's smart MiniMed Paradigm® insulin pumps and Guardian® REAL-Time continuous monitoring systems.

4.11. Cygnus Inc

Founded in 1985, the Cygnus' GlucoWatch® Biographer provides automatic and non-invasive measurement of glucose levels from fluid between the skin tissues (http://www.cygn.com/homepage.html). However, the company had an arbitration matter with Johnson and Johnson and terminated all activities in 2003 followed by the sale of its glucose-monitoring assets to Animas Corporation and Animas Technologies LLC in 2005. However, Animas was no longer selling the current model GlucoWatch G2 Biographer system, effective July 31, 2007. The company will continue to sell AutoSensors and provide customer support for the GlucoWatch system through July 31, 2008 (http://www.glucowatch.com).

4.12. Neogen Corporation

Neogen Corporation (http://www.neogen.com) provides a diverse range of products dedicated to diagnostic testing for food and animal safety. Its GeneQuence Automated System is a fully automated 4-plate processing system for detection of pathogens. GeneQuence utilizes a novel DNA hybridization technology which assays for *Salmonella*, *Listeria* spp., *Listeria monocytogenes*, and *E. coli* O157:H7. Each test kit uses two specific DNA elements ensuring the highest of specificity, thereby increasing the confidence of the results (1–5 CFU/25g sample), which are obtained in less than 2 h. The automated plate handling unit makes it possible to test more than 700 samples in an 8 h work day with very little hands on time. The AccuPoint ATP Sanitation Monitoring System provides sanitation monitoring capability in a hand-held unit. The company also supplies ELISA test kits/reagents and testing equipment for foodborne bacteria, drug residues, toxins, and biologically active substances. Recently (March 14, 2008), Neogen has received approval for the new United States version of its quick and easy BetaStar® test for dairy antibiotics in milk. The BetaStar® US test (AOAC-RI No. 030802) is an extremely simple dipstick test that detects dairy antibiotics in the beta-lactam group, requiring only minimal training and equipment to produce consistently accurate results.

4.13. Panbio diagnostics

Technical platforms of this Australian company include the enzyme-linked immunosorbent assay, indirect fluorescent antibody test and rapid lateral flow devices (http://www.panbio.com.au). Panbio activities focus on West Nile virus, Japanese encephalitis, leptospirosis and malaria. The company has two major technology platforms: homogeneous immunoassays and oligo rapid immunochromatography.

4.14. Applied biophysics

This company has commercialized an impedance microarray system for probing cells and cell behavior including cell adhesion and proliferation, cytotoxicity, tumor invasion, wound healing, etc. (http://www.biophysics.com). The core technology is the measurement of the change in impedance of a small electrode (250 μm in diameter) microfabricated on the bottom of tissue culture wells and immersed in a culture medium. The attached and spread cells act as insulating particles because of their plasma membrane to interfere with the free space immediately above the electrode for current flow, resulting in a drastic change in the measured impedance. Cell densities ranging from a heavy confluent layer to very sparse layers can be measured with this approach. The technique is sensitive enough for detecting even a single cell. The technology was invented by Ivar Giaever, a Nobel Laureate in Physics.

4.15. The Spreeta (Texas instruments) and other SPR biosensors

This company commercializes compact, low-cost and commercially available SPR-based sensors (http://www.sensatatechnologies.com/files/spreeta-tspr2kxy-product-bulletin.pdf). The units consist of a near-infrared diverging LED light source, a polarizer, a gold sensing layer, a reflecting mirror and a photodiode-array detector. The polarized light is emitted toward the gold sensing surface and reflected at different angles. At certain angles of light incidence, resonance of the gold surface plasmons occurs and the intensity of the reflected light drops dramatically. The light is reflected on a mirror and projected onto the photodiode array where the light intensity is measured. The position of the light intensity minimum is extremely sensitive to changes in refractive index (RI) of the fluid in the sensing area. Therefore, RI changes near the sensing area can be measured by monitoring the light intensity minimum shift over time. However, the Spreeta technology might not be as sensitive as the standard ELISA procedure (Spangler et al., 2001). SensiQ with a dual channel is a state-of-the-art data analysis tool to provide kinetic, affinity and concentration data researchers can use with a high degree of confidence. In 2008, the manufacturer of SensiQ (ICx Nomadics Bioinstrumentaion Group, Oklahoma City, OK) just launched SensiQ Pioneer, a fully automated SPR platform while maintaining the cost affordability (http://www.discoversensiq.com). XanTec Bioanalytics GmbH of Germany is another company that commercializes SPR biosensors (http://www.xantec.com). Notice that the coatings of its sensor chip are claimed to be robust and prevent exposure of hydrophobic nanodomains or pinhole defects which can cause non-specific interactions.

5. Trends and future possibilities

The increasing demands and interests in developing implantable glucose sensors for treating diabetes has led to notable progress in this area, and various electrochemical sensors have been developed for

intravascular and subcutaneous applications. However, implantations are plagued by biofouling, tissue destruction and infection around the implanted sensors and the response signals must be interpreted in terms of blood or plasma concentrations for clinical utility, rather than tissue fluid levels (Li et al., 2007). In view of technical feasibilities and challenges, there is greater success in developing hand-held biosensors than implantable devices.

There is also great interest in parallel, high-throughput assays for clinical, environmental, and pharmaceutical applications. This requirement has paved the way for the development of integrated miniaturized devices to reduce the development and production costs, particularly for the applications that require cost-exorbitant biological materials. In this context, the development of disposable biosensors has received a great deal of interest for the detection of biological agents/toxins (Spichiger-Keller, 1998)). One of the key steps in the construction of such miniaturized electrochemical sensors is to select a pertinent method for probe immobilization. For example, the use of an electropolymerized conducting polymer as matrix to immobilize the biorecognition probe is of particular interest. The electrosynthesis of conducting polymers allows for precise control of probe immobilization on surfaces regardless of their size and geometry (Dong et al., 2006). Since the polymerization occurs on the electrode surface, the probes are essentially entrapped in proximity to the electrode. This feature is of particular importance toward the development of sensing microelectrodes and microelectrode arrays to shorten the response time and alleviate interference from the bulk solution. Furthermore, the amount of immobilized probes can be easily controlled either by changing their concentration or by adjusting the thickness of the polymer matrix through the electrode potential, electropolymerization time, or both. Of particular interest is the use of an electropolymerized pyrrolepropylic acid film with high porosity and hydrophilicity to covalently attach protein probes, leading to significantly improved detection sensitivity compared with conventional entrapment methods (Dong et al., 2006).

Besides conventional electrode materials such as platinum, gold, silver, glassy carbon, etc., novel electrodes fabricated from diamond doped with boron to extend the overpotential has emerged, particularly for monitoring arsenic in drinkable water (Hrapovic et al., 2007). Nanomaterials such as carbon nanotubes together with nanoparticles (gold, platinum, copper, etc.) have been reported to significantly enhance detection sensitivity and facilitate biomolecule immobilization. Such combined materials also promote electron-transfer reactions between the active sites of the enzyme and the detecting electrode. Notice also that selective and sensitive electrochemical detection of glucose in neutral solution becomes feasible using platinum–lead alloy nanoparticle/carbon nanotube nanocomposites. The recent bloom of nanofabrication technology and biofunctionalization methods for carbon nanotubes (CNTs) has stimulated significant research interest to develop CNT-based biosensors for monitoring biorecognition events and biocatalytic processes (Luong et al., 2007). CNT-based biosensors could be developed to sense only a few or even a single molecule of a chemical or biological agent. Aligned CNT “forests” can act as molecular wires to allow efficient electron transfer between the detecting electrode and the redox centers of enzymes to fabricate reagentless biosensors. Electrochemical sensing methods for DNA can greatly benefit from the use of CNT-based platforms since guanine, one of the four bases, can be detected with significantly enhanced sensitivity. CNTs fluoresce, or emit light after absorbing light, in the near near-infrared region and retain their ability to fluoresce over time. This feature will allow CNT-based sensors to transmit information from inside the body. The combination of micro/nanofabrication and chemical functionalization, particularly nanoelectrode assembly interfaced with biomolecules, is expected to pave the way to fabricate improved biosensors for proteins, chemicals, and pathogens. However, several technical challenges need to be overcome to tightly integrate CNT-based platforms with sampling, fluidic handling, separation, and other detection principles. The majority of biosensors reported in the literature require various cleaning/washing steps, separately from the detection process. Furthermore, many detection schemes require the addition of extra reagents including co-enzymes, redox species, etc. to generate a detectable product.

The optical sensor deserves a revisit here because of the recent development of fluorescent nanocrystals (quantum dots) and significant progress in photonics. Quantum dots are brighter than molecular dyes, resistant to photobleaching, and amenable to multiplexed detection by controlling the size of the fluorescent nanocrystals to tune the fluorescence wavelength (Bruchez et al., 1998). Nanoparticles can be used to provide nanoprobes for imaging and sensing for early detection of diseases. Nanophotonics deals with manipulation of optical excitation and dynamics on a nanoscale, opening opportunities for many optical and optoelectronic technologies including biosensing. Nanoplasmonics is an area of nanophotonics that deals with optically generated interfacial electromagnetic excitations in metallic nanostructures. Nanomagnetics deals with control, manipulation and utilization of magnetic interactions on nanoscale. Such promising and emerging technology might also provide solutions to the obstacles that impede successful commercialization of biosensors. Gold nanoparticles containing DNA “barcodes” may provide that next generation technology (Stoeva et al., 2006). Biocodes consist of nucleic acid sequences of 30 to 33 bases. Part of each biobarcode recognizes a specific target DNA sequence, while the remainder of each biobarcode is common among all barcodes and is necessary for detection and readout functions in the assay. Each biobarcode is linked to a 30-nanometer-diameter gold nanoparticle. The researchers also constructed magnetic microparticles containing a short piece of DNA that binds to a separate unique region of the target DNA. Optical biosensors could become a powerful tool in the imminent future for the real-time and remote detection of emerging infectious diseases (Monk and Walt, 2004). As high-end instruments, the SPR array equipped with auto-samplers and powerful data acquisition continues to play an important role in the most profitable pharmaceutical and biotechnology companies to speed up the drug discovery and development process. Current technical achievements in SPR microarray will lead to compete against application of immunoassays, a workhorse widely used for determination of numerous important substances.

The biosensing platform must function well in a real-world sample environment where selectivity, sensitivity, detection limits, and ruggedness are the four prerequisites. Complex clinical and environmental samples often impede accuracy, sensitivity and the lifetime of the sensor due to cross cross-reactivity, inhibition of the detection method, and non-specific adsorption of unwanted species in the sample. The use of CNTs in biosensing looks very promising as reflected by some significant patents in this area and other research and development endeavors. However, nanostructure-based biosensors could be relatively expensive, with high development and manufacturing costs for the immediate future. It is still uncertain if the increased capability of nanosensors is sufficient to open up large markets, and quickly engendering a rapid decrease in costs. The biosensor has a tremendous potential for the detection of microbial contamination in foodstuffs and the microarray technology can simultaneously and easily detect up to 12 different pathogens. Common bacteria found in meat are *Salmonella*, *E. coli* 0157:H7, generic *E. coli*, *L. monocytogenes*, *Campylobacter jejuni* and *Yersenia enterocolitica* (primarily in red meat). All of these pathogens are associated with stomach illness in human beings. Besides the protection of consumers, food producers can make decisions more quickly about applying treatments such as antiseptics treatment, cooking operations to kill the pathogens and modification of their sanitation plans.

The biosensor industry is dominated by a few large multinational companies with enormous sources of finance for technology acquisition and validation. The market entry for a new venture is very difficult unless a niche product can be developed and the company must have vast financial resources for technology development, demonstration, validation, and marketing. An example for a potential niche product is the development of an autonomous system, disposable, low-cost and requiring no external equipment, reagents, or power sources. In this context, of interest is a simple method for patterning paper to create well-defined, millimeter-sized channels, comprising hydrophilic paper enclosed by hydrophobic polymer for the analysis of both glucose and protein urine samples (Martinez et al., 2007). Although it only detects glucose at high concentrations (~2.5 mM), chemistry can be improved and adapted for other important clinical and environmental samples. Another niche market is the rapid and sensitive detection of biological agents that harm people, livestock, or plants. The key issue is trace detection in a short time (<1 min) since small amounts of pathogens can cause illness and releases can be diluted rapidly in the environment. For example, in the food industry or clinical samples, a detection limit of 1 pathogen/g or 1 pathogen/ml is desired. Even with thousands of analytes per pathogen, the required detection limit is 1.7 aM (1.7×10^{-18} M), still a real challenge in analytical chemistry. The U.S. Food Safety Inspection Service has established a zero-tolerance threshold for the most fearful strain *E. coli* O157:H7 contamination in raw meat products (Jay, 2000). The infectious dosage of *E. coli* O157: H7 is ten cells whereas the Environmental Protection Agency standard in water is 40 cells/L (Dubovi, 1990). Therefore, the biosensor system must include sample collection and sample preparation, biodetection (often using multiple biosensors), data integration and analysis, and finally reporting of the results. Consequently, the system tends to be costly and complicated. Novel approaches are under development to miniaturize such integrated system to minimize consumables, analysis time and improve reliability. The development of microscale separation devices, particularly micromachined capillary electrophoresis chips coupled with amperometric detection, has received significant attention in recent years (Fischer et al., 2006). Integration of a miniaturized biosensor with a separation scheme will continue to be a subject of intensive investigation.

Toxicologic information of drugs, pollutants, toxins, nanomaterials such as quantum dots and nanoparticles should be established to protect human health and environmental integrity. A recent report indicates that long straight carbon nanotubes may be as dangerous as asbestos fibers (Poland et al., 2008). They might cause cancer in cells lining the lung, a pilot study with mice. Nanotubes under twenty micrometers, and long nanotubes which are tangled up into balls, do not cause asbestos-like problems. Although much more work will be required to provide definitive proof, however, considering the terrible effects of asbestos that emerged in the 1960s, researchers are urging caution, particularly for the use of CNTs and other nanomaterials in biosensing, bioimaging, and drug delivery. This is of utmost importance because carbon nanotubes have been advocated for a wide range of products under the assumption that they are no more hazardous than graphite. While annual global spending on nanotechnology research is about 9 billion dollars, only 39 millions are invested in the analysis of the safety of nanomaterials in human and the environment. In this context, cell-based impedance spectroscopy has emerged as one of the potential candidates (Xiao and Luong, 2003) and this system has been adapted for providing cytotoxicity information of quantum dots and other nanomaterials (Male et al., in press). This application could be a niche market for cell-based assays because of their broad applicability for the detection of both known and unknown chemical agents and bioagents. Lastly, attention should be paid to a new class of affinity proteins, so-called affibodies (Nord et al., 1995; Nygren, 1997). Despite their smaller size and simpler overall structure, these proteins have binding features similar to antibody variable domains in that selective binding with high affinity can be obtained towards various target molecules (Hansson et al., 1999). Such features make them interesting alternatives to antibody fragments for use as recognition units in larger fusion proteins for therapeutic, diagnostic and biosensing applications, a virtually unexplored field. It will remain to be seen whether biosensor technology with novel biorecognition elements can make any breakthrough towards the development of rapid and reliable detection for mad cow disease, a problem which has been waiting for a right solution.

6. Conclusion

The development of ideal biosensors which are fast, easy to use, specific, and inexpensive, doubtlessly, requires the significant upfront investment to support R&D efforts and this is a key challenge in the commercialization of biosensors. To date, progress in biosensor development is somewhat incremental with low success rates and there is the absence for huge volume markets except for glucose sensors. The future trend includes the integration of biosensor technology with leading-edge integrated circuit, wireless technology and miniaturization. However, one must carefully look at the special demands of analytical chemistry and technology feasibility prior to any decision making or commitment to undertake a new research project or development. From a technical viewpoint, a dream biosensor might be a combination of SPR with electrochemical detection to process “real-world” samples such as blood serum, environmental samples and other colored samples.

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