SESSION 2

ELECTRO-CHEMICALLY BASED SENSORS
# Electrochemiluminescence Based Micro-Array Systems for Biochemical Assays and Detection of Biological Agents

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### Technology

- Electrochemiluminescence is a well-established process in which certain chemical compounds emit light when electrochemically stimulated.
- Multi-Array™ Technology combines electrochemiluminescence detection with arrays and rapid, parallel readouts.
- Screen printed electrode arrays with carbon biosurfaces are manufacturable, reproducible and disposable.

### Existing Capabilities

- Established platforms for pathogen detection, clinical applications, and biochemical assays.
- Array based detection systems for multi-analyte detection of proteins, nucleic acids and cells.
- Assays with high sensitivity, low background, simple formats.
- Compatibility with complex samples (blood, food, environmental samples).
- Operation at ambient conditions with modest infrastructure.
- Robust, highly reproducible measurements.

### Emerging Developments

<table>
<thead>
<tr>
<th>Bacillus anthracis</th>
<th>Yersinia pestis</th>
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</thead>
<tbody>
<tr>
<td>Vaccinia Virus</td>
<td>Clostridium botulinum</td>
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</table>

- Fast, portable and quantitative systems for:
  - Clinical and environmental samples
  - Antibody and nucleic acid based assays
  - Detection of bacteria, viruses and toxins
  - Performance that matches clinical analyzers
  - Disposable cartridges

### Labels and Detection

- Assays require measurable response
- Specific binding assays label
- Labels provide signal to measure
- Measured signal used to quantitate analyte
- Labels distinguished by signal detected.

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[Image of electrochemiluminescence (ECL) based micro-array systems for biosensing, labels and detection diagrams.]
2. Electro-Chemically Based Sensors

Specific Binding Assays

Electrochemiluminescence (ECL)

A well established process in which certain chemical compounds emit light when electrochemically stimulated

\[ \text{Electro-} \quad \text{chemi-} \quad \text{luminescence} \]

\[ \text{electrochemically driven} \quad \text{chemical energy} \quad \text{emitting light} \]

\[ N \xrightarrow{\text{REDOX}} N^* \xrightarrow{} N + hn \]
Electrochemiluminescent Labels

- Ruthenium chemistry confers many advantages:

<table>
<thead>
<tr>
<th>Property</th>
<th>Advantage</th>
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<tbody>
<tr>
<td>~1000 Daltons</td>
<td>Retains bioactivity of labeled compounds</td>
</tr>
<tr>
<td>Stable for years</td>
<td>Long shelf life, high accuracy</td>
</tr>
<tr>
<td>Aqueous &amp; organic-soluble</td>
<td>High compatibility w. assays &amp; reagents</td>
</tr>
<tr>
<td>Hydrophilic</td>
<td>Low non-specific binding</td>
</tr>
<tr>
<td>Low reactivity</td>
<td>Compatible with cells, proteins, nucleic acids, small molecules.</td>
</tr>
<tr>
<td>High specificity</td>
<td>Low background; reduced interference</td>
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Ruthenium (II) tris-bipyridine NHS ester

**R Group**
- NHS Ester
- Hydrazide
- Amine
- Maleimide
- Phosphoramidite

**Target Molecule**
- Amines
- Carbohydrates
- Carboxylic Acids
- Thiols
- Oligonucleotides

Electrochemiluminescence Process
2. Electro-Chemically Based Sensors

**Electrochemiluminescence: Optical Detection**

- Light proportional to concentration.
- Current does not change and cannot be used to measure the analyte.

**Electrochemical Detection vs. Electrochemiluminescence**

- Electrochemical reactions at electrode
- Material on electrode
- Electrochemiluminescence
**Electrochemiluminescence: Commercialization**

- IGEN International, Inc.
  - Founded 1983
  - Developed ORIGEN technology, a detection system based on electrochemiluminescence
  - Manufactures and markets products based on ORIGEN technology
  - Holds Key Patents

- Meso Scale Discovery
  - Founded 1995
  - Division of Meso Scale Diagnostics, LLC., a joint venture involving IGEN International, Inc.
  - Multi-Array Technology

**Selected Patents and References**


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**IGEN’s ORIGEN Technology: Bead Based Systems**

Solid phase assays employing magnetic particles

Measurements carried out using reusable flow cell
2. Electro-Chemically Based Sensors

ORIGEN Technology: Clinical Applications

Roche Elecsys® Systems

• Over 7500 clinical instruments placed
• Over 50 clinical assays developed
• Over 100,000,000 clinical determinations carried out

Elecsys® 2010
High Throughput, Continuous Random Access Benchtop Immunoassay Analyzer

Elecsys® 1010
Sample-Selective Multibatch Benchtop Immunoassay Analyzer

ORIGEN Technology- Pathogen Detection

Biological Agents

<table>
<thead>
<tr>
<th>Agent</th>
<th>Sensitivity</th>
</tr>
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<tbody>
<tr>
<td>Staphylococcal enterotoxin B</td>
<td>0.5 pg/mL</td>
</tr>
<tr>
<td>B. Anthracis</td>
<td>1 x 10⁹ cfu/mL</td>
</tr>
<tr>
<td>Botulinum A toxin</td>
<td>4 pg/mL</td>
</tr>
<tr>
<td>Cholera toxin B</td>
<td>2 pg/mL</td>
</tr>
<tr>
<td>Ricin A chain</td>
<td>0.5 pg/mL</td>
</tr>
<tr>
<td>B. Subtilis</td>
<td>5 x 10⁹ cfu/mL</td>
</tr>
<tr>
<td>E. Coli O157:H7</td>
<td>0.1 x 10⁹ cells/mL</td>
</tr>
<tr>
<td>MS2 (coliphage)</td>
<td>1 x 10⁹ pfu/mL</td>
</tr>
<tr>
<td>Salmonella</td>
<td>1 cfu per 100 cm² of surface</td>
</tr>
<tr>
<td>Listeria</td>
<td>1 cfu per 100 cm² of surface</td>
</tr>
<tr>
<td>Cryptosporidum parvum oocytes</td>
<td>1000 oocytes</td>
</tr>
<tr>
<td>Campylobacter</td>
<td>10,000 cfu per g of stool</td>
</tr>
</tbody>
</table>

Sample Matrices

• Clinical – Plasma, serum, blood, feces
• Food – Fish, chicken, ground beef, dairy, juice
• Environmental - Soil, air, water filtrates, surface swabs, plant extracts

Examples taken from Yu et al.; Biosens. Bioelectron., 14, 829-840 (2000) or provided courtesy of IGEN International
**ORIGEN Formats for Assays**

**Immunooassays**

**Enzymatic Activity Assays**

**Binding Assays**

**Nucleic Acid Detection Assays**

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**Meso Scale Discovery (MSD)**

**Arrays**

**Electrochemiluminescence**

**Discovery**
2. Electro-Chemically Based Sensors

**Arrays and Detection**

**Arrays**
- Labels bound to surface
- Small amount of label
- Low light levels
- High background regions
- Multiple simultaneous assays
- Patterns
- Organization
- Low overheard (minimal fluidics)

**Electrochemiluminescence Detection**
- Surface driven excitation
- High efficiency process
- Signal amplification
- Selective excitation
- Parallel detection
- Convenient spatial localization
- Identification of discrete locations
- Tolerant of no-wash formats

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**Multi-Array Technology: Screen Printed Electrodes**

**Patterned Electrode Setup**
- Working electrode
- Counter electrode
- No reference electrode

**Carbon Ink Electrode Advantages**
- Flexible formats
- Binding capacity ~10x higher than polystyrene
- Suitable properties for electrochemiluminescence
- Direct binding of Avidin, BSA, IgG, peptides, DNA, membrane fragments.
Multi-Array Technology: Multi-Spot Plates

Advantages of printed patterned electrodes:
- Assay Panels
- Multiplexing
- On-board standards
- Higher throughput
- Less sample
- Reduced Noise

Multi-Array Surface Chemistry
- Carbon electrodes
- Novel surface chemistries
- Derivatization schemes for patterning
- Flexible chemistry for panels
Multi-Array Microplates

- 96-well plate
- 384-well plate
- 1536-well plate

Applications
- Multi-Analyte Assays
- Genomics and Proteomics
- Multi-parameter testing

Multi-Spot Array Plates

- Plate sector
- Applications
  - Multi-Analyte Assays
  - Genomics and Proteomics
  - Multi-parameter testing

- 96 wells: 4, 7, and 10 spots/well
- 24 wells: 25, 64, and 100 spots/well
Multi-Array Technology: Imaging Detection

Sector HTS: Features

- Custom optics with telecentric lens and cooled CCD camera imaging
- High Speed 2D motion control
- Compatible with 96-, 384-, 1536-well and 24-, 96-Multi-Spot custom plates
- Processing Speed: 1 plate per minute, format independent
- Fully robotically compatible
- Integrated plate stacker
- Built-in bar code reader
- No-wash assay formats
2. Electro-Chemically Based Sensors

## Sector HTS Performance

*Graph showing signal/background values for one and four spots.*

- Sensitivity ~ $10^{-1}$-$10^{-6}$ molecules
- Dynamic range > 4 orders of magnitude
- Smaller sometimes better

## Sector PR: Features

- Custom optical design with photodiode array for parallel measurements
- Electrochemiluminescence detection
- Reads 96-well custom plates
- Processing speed: ~ 2 minutes/plate
- Manual and Robotic interfaces
- Assay chemistry transferable to companion Sector HTS

*A highly reflective plate is used to maximize light collection efficiency.*
Multi-Array Applications: Complex Assays

- Pathogen detection
- Assays in complex samples
- Assays with whole cells
- Multi-analyte assays
- Nucleic acid binding assays
- Immunoassays in cell lysates
Multi-Spot Applications: Cytokine Panel

Multi-Spot Applications: Cytokine Panel

- Detection limits: 1-10 pg/mL
- Linear range extends up to >3000 pg/mL
- Calibrators are in RPMI media containing 10% human serum
- 25% above background signal
- Method is scalable to larger arrays
### Multiplex Cytokine Assays with Whole Cells in a Single Well

- **Procedure:**
  - Add whole blood diluted 1:10 in RPMI
  - Add LPS
  - Incubate 4 h at 37°C
  - Add detection Ab. 1 h at 4°C
- **Measure:**
  - Measure on Tecan HTS Reader

- **Results:**
  - Cells activated in polypropylene plate
  - Cells activated and analyzed in MSD Multi-Spot Plate

### Multi-Array Applications: Pathogen Assays

**Influenza A Antigens Detection**

- Group A capture (Spot 4)
- RSV capture (Spot 2)
- Flu A capture (Spot 1)

**Influenza B Antigens Detection**

- Group A capture (Spot 4)
- RSV capture (Spot 2)
- Flu B capture (Spot 2)
- Flu A capture (Spot 1)

**RSV Antigens Detection**

- Group A capture (Spot 4)
- RSV capture (Spot 2)
- Flu A capture (Spot 1)

**Influenza A Antigens Detection**

- Group A capture (Spot 4)
- RSV capture (Spot 2)
- Flu A capture (Spot 1)
2. Electro-Chemically Based Sensors

Summary of Key Features

Multi-Array Plates
- Direct capture on a carbon electrode
- Single-use microplates
- High-throughput format
- Spatial multiplexing
- Parallel readout

ECL Detection
- High sensitivity – low background
- Large dynamic range
- Small label
- Versatile coupling chemistries
- Stable reagents
- Spatial and temporal control
- Diverse assay portfolio

Rapid Detection of Agents of Biological Threat

Proposed System Features

Assays
- Rapid measurement – 10 minutes
- Detection of multiple analytes (up to 16)
- Agents: viruses, bacteria, toxins
- Targets: proteins, nucleic acids, small molecules
- Sensitive, quantitative detection based on ECL
- Possible samples: blood, plasma, throat swab, water, food, soil

Cartridge and Reader
- Disposable cartridges containing all reagents
- Small sample volume (15 – 150 μL)
- Built-in controls
- Portable, battery-operated reader

Agents: Bacillus anthracis, Yersinia pestis, Vaccinia Virus, Clostridium botulinum
Leveraging Multi-Array Technology

Technology Base
- Electrode array fabrication
- Screen-printed carbon inks
- Surface coatings & reagent immobilization
- ECL chemistry
- Multi-analyte detection
- Broad menu of assays
- Detection electronics

Development
- Miniaturized field unit
- Fluidics
- Sample processing
- Reagents contained in cartridge
- Specific assay panels

Parallel Development Path

Assays in Multi-Array Plates
- Identify Pathogen Targets
- Acquire and Screen Antibodies
- Develop and Optimize Assays
- Test Matrix Effects
- Develop Dry Reagents
- Demonstrate Performance and Stability
- Combine Assays into Panels

Reader
- Develop Specifications/Requirements
- Select and Test Components
- Design and Prototype Subsystems
- Build Integrated Prototype

Cartridge
- Design and Print Electrode Array
- Design Plastic Fluidic Manifold
- Develop Assembly Methods

Integrated Assay Platform
- Transfer Assay Panels to Cartridges
- Optimize Fluidic Operations in the Cartridge
- Validate Assay Performance
**BIO/CHEMICAL SENSING USING THIN FILM RECOGNITION ELEMENTS**

*Richard M. Crooks*

**INTRODUCTION**

There are three broadly implemented approaches for sensing the presence of biological agents (biologics). The first relies on the use of a second, complementary biologic that is either immobilized on a surface, dispersed in solution, or both (as for proteins immobilized on soluble colloids or beads). Specific applications include DNA gene chips, in which target DNA binds to its surface-immobilized complement, and ELISA assays. The second biosensing strategy relies on target recognition by synthetic templates. These template replicas are often cast from the original target. A well-known example of this approach is polymer imprinting,\(^1\,^2\) in which a polymer precursor and a biologic are mixed and then polymerized. Removal of the target from the polymer leaves behind a replica into which the target is able to rebind. These first two-biosensing approaches rely on highly specific interactions between the receptor and the target to provide selectivity. The third biosensing strategy relies on a different principle: separation or a mixture of biologics followed by detection. In this case selectivity is provided principally by the separation rather than complex formation.

This review focuses on the use of molecular recognition elements contained within thin films to sense the presence of biologics. The word "biosensor" implies that a biologic is involved in the sensing event either as the target or the probe. Here we focus on the family of biosensors that employs either a biologic or a synthetic chemical (such as a polymer) as the receptor for detection of a biological target. Other biosensors use biologics to sense the presence of organic chemicals, but these are not discussed here.

Thin-film biosensors can be divided into three categories.

- Monolayers of biologics attached onto the surface of a solid support (either a monolithic surface or the surface of a soluble or suspended small particle).
- Organized multilayers that are composed wholly or in part of a biological recognition element.
- Disorganized, porous films that contain a biologic; these include polymers, sol-gel-derived materials, and the like.

**UNIFYING CONCEPTS**

There are five major issues that must be addressed during the design phase of biosensor fabrication: sensitivity, selectivity, stability, flexibility, and cost. The first three of these are largely scientific problems and the last two are engineering or business issues. Recognizing that it is, to some extent, an exercise in futility to discuss the design of a biosensor without specifying the target, it is possible define some unifying concepts that are common to most biosensor designs. First, biologic recognition elements often provide a high level of selectivity, so when these materials are used selectivity is often not a major concern. Second, biological materials, such as cells and proteins, are inherently fragile, and therefore development of methods for engineering robustness into sensor design is of critical importance. It is worth mentioning that the relative importance of selectivity and robustness for biosensors is essentially reversed for chemical sensors. There are three primary failure modes when biologics are used within thin-film-based biosensors:
denaturation or death (depending on the specific biologic), desorption, and contamination. The latter is particularly problematic for implantable sensors or those used for detection in untreated samples. The final three unifying issues, sensitivity, flexibility (the absolute number of problems that the sensor design can address), and cost depend too heavily on the specific sensing problem to address in a general way. It is obvious, however, that if the design is specific for a single analyte and the market is limited, as for many military applications, there will not be very much commercial interest (writing a business plan that relies on the government as the main customer is usually not very attractive for investors). Another way of saying this is that sensors for targets that are unlikely to be important (such as biological weapons) are very likely to enthrone investors, who are mainly interested in products for existing markets (examples include the home pregnancy kit and the recently announced HIV-testing kit, both of which are one-shot assays that sell for about $20).

**MONOLAYER AND BILAYER COATINGS**

Biosensors based on monolayer films have been around for a long time and their advantages and disadvantages are well understood, although specific details depend on the particular support and monolayer film used. For sensors based on protein monolayers, orientation of the protein, so that the active site projects away from the substrate, is a key issue. If the structure of the protein is known, then this can be accomplished by attaching specific amino acids within the protein to the surface. If such residues are not available, they can be engineered into the protein. Denaturation is also a key issue that is driven by strong interactions between hydrophobic surfaces and the hydrophobic interiors of proteins. These can be overcome by supporting the protein on a biocompatible layer (for example a hydrophilic polymer or phospholipid membrane) or tethering it above a biocompatible surface. Orienting and stabilizing proteins on surfaces (that is, maintaining their activity at its bulk value) is generally a poorly understood and unsolved problem.

Monolayer coatings provide direct access (rapid mass transport) of the target to the recognition site. That is, the target need not negotiate a series of small pores to locate the receptors. This, along with ease of fabrication and characterization, are the principal virtues of monolayer thin films. However, this direct exposure of an active site to the analysis solution may also provide a clear pathway for contaminants to block receptors, thereby rendering the sensor useless. The only way to address this problem is to clean up the sample prior to exposing it to the receptors. This added complexity is probably best accomplished using the type of integration provided by microfluidic systems. For non-protein bioreceptors the situation might be somewhat different. For example, monolayer DNA arrays, which rely on direct attachment of probe DNA to a surface, do suffer a form of mass transfer problem. Specifically, the DNA "active site" does not have a well-defined shape or orientation relative to the substrate (in fact, neither of these is well understood at present). This means that targets may be slow to hybridize. One solution to this problem is to reduce the number density of DNA probes immobilized on the surface, but this reduces sensitivity and therefore may require a higher surface area per DNA pixel.
A final key aspect of monolayer arrays is that they can be relatively straightforwardly patterned using a variety of lithographic approaches. An example of a patterned monolayer of macrophage cells is shown in Figure 1. Patterning was carried out by a combination of microcontact printing and polymer grafting. The result is an two-dimensional array of 60 µm square "biopixels" or "corrals" (as we call them in Texas) that each contain one or more viable cells (the exact number depends on how long the cells have been cultured on the pattern, the size of the cells, and the size of the corrals). Figure 1a shows a poorly designed set of corrals in which the cells are able to span the walls between corrals. Figures 1b shows a properly designed surface in which all the cells are confined within their corrals. The close-up in Figure 1c shows that up to nine cells can be confined within a single corral. Bioarrays such as this provide a means for analyzing either the cells themselves or analytes that may affect the cells in a detectable way.

In recent years it has been shown that fluid phospholipid membranes can be immobilized onto planar supports and even patterned into discreet arrays (Figure 2). This is an important advance because it provides a means for using membrane proteins, which account for about half of all proteins known, as biosensing elements. Besides greatly expand the number of potential recognition elements for use in biosensors, it provides the two additional benefits. In Figure 3, the pore-forming protein α-hemolysin has been inserted into a suspended phospholipid bilayer. This example is important because it illustrates the idea of detecting transport events rather than binding between a receptor and target, which is the phenomenon upon which nearly all existing thin-film biosensors are based. This in turn provides an opportunity for implementing stochastic sensing, in which analytes are detected one at a time. For applications aimed at detecting slight differences (size, charge, etc.) between otherwise similar molecules, stochastic sensing may be more useful than methods that provide average information about an ensemble of targets. The second advantage of using fluid bilayers as hosts for receptors is that it may take advantage of the multivalency that many biological targets display. The challenge to using fluid phospholipid bilayers in real biosensors relates to the robustness of the bilayer: specifically, its electrical resistivity, the length of time it remains intact, and the types of substrates on which such bilayers can be formed. These are all active areas of research at the present time, and the advantages of stochastic sensing and detection via multivalency will doubtless be strong drivers for development of...
In addition to monolithic substrates, monolayers of receptors can also be attached to soluble, suspended, or trapped nanometer- or micron-scale particles. Figures 4 and 5 provide examples of using this approach. Figure 4 shows an example of a biosensor based on DNA monolayers attached gold nanoparticles. This is a versatile approach to biosensing, because the DNA receptors provide specificity and the gold nanoparticle are both the scaffold and the transducers that report hybridization via a color change. An additional feature of this system is that signal transduction relies on cooperativity between many nanoparticles. This provides a kind of amplification of the recognition event, similar to that provided by recognition schemes that result in formation or activation of catalysts (such as some ELISA-based methods). Viable demonstrations of biosensors that take advantage of amplification and cooperativity have started to appear in greater number in recent years, and they will surely be of critical importance in future years.

Figure 5 shows that biosensors can be based on much larger particles, in this case insoluble, surface-modified polystyrene beads, entrapped within a microfluidic device. For example, a monolayer of enzymes or DNA can be attached to such beads prior to their being introduced into the microfluidic system. This approach provides a few significant benefits compared to modification of monolithic substrates. First, bead chemistry is very well developed, and therefore many of the tools of synthetic chemistry can be straightforwardly adapted to bead-based biosensors. Second, bioreceptors immobilized on beads can be characterized using tools, such as NMR, that are not useful for studying monolithic supports. Third, beads modified with different coatings can be immobilized in different reaction chambers to perform multiple analyses on a single sample. For example, in the experiment illustrate in Figure 5, glucose oxidase is immobilized on the beads in the first microreactor and horseradish peroxidase (HRP) is attached on the beads in the second microreactor. When glucose is introduced into the microfluidic system, it is converted into gluconic acid and peroxide in the first reactor. Subsequently peroxide, along with nonfluorescent amplex red from a side channel, is introduced into the second chamber, where HRP catalyzes the amplex red to fluorescent resorufin, which is easily detected.

POLYMER THIN FILMS

Organic polymers can be used to entrap biological recognition elements, to prepare synthetic templates for recognizing biomolecules, to prevent adsorption of contaminants, and to connect molecular receptors to transducers. A vast number of polymer types have been used for these purposes. For example, an electrochemical glucose sensor has been reported that encapsulates glucose oxidase within a redox polymer. In this scheme the polymer provides two functions. First, it provides a biocompatible scaffold for the enzyme so that it does not denature. Second, it provides a means for shuttling electrons between the underlying electrode and the redox enzymes that convert glucose to gluconic acid and peroxide. The idea of using polymers, or polymer composites, that carry out multiple functions is certain to be an important area of research during the coming decade.
An example of how composite films can be used to provide multiple sensor functions, in this case improving robustness and selectivity, is shown in Figure 6. Here, a structured composite film consisting of an active “chemical filter” resides atop a polymer layer that contains the receptor elements (in this case cyclodextrins). The overlayer, which in this case is a polyamine, prevents fouling of the receptors and also prevents positively charged interferents from accessing the receptors. More sophisticated synthetic means for preparing more sophisticated multifunctional composites such as these will be important in coming years.

Hydrogels are another type of organic thin film that is useful for biosensing applications. We recently showed, for example, that these materials can be photopolymerized within microfluidic systems, that they are able to act as passive valves in microfluidic-based biosensors, and that they are biocompatible hosts for cells and enzymes. For example, Figure 7a shows an optical micrograph of a hydrogel micropatch photolithographically defined and co-polymerized with bacteria within a microfluidic channel. Pores penetrating the hydrogel are sufficiently large (~ 1 nm) that small molecules can freely diffuse into it, but they are small enough that bacteria (~ 1 µm) or enzymes (~5 nm) are entrapped. We were able to show that the cells were viable following entrapment, that the cellular machinery could perform chemical reactions on analytes that penetrate both the hydrogel and the cell wall, and that the cells could be lysed within the hydrogel (Figure 7b).

INORGANIC THIN FILMS

Several inorganic thin films have been found to be effective platforms for biosensing. For example, a porous silicon-based optical interferometric biosensor has been reported. The sensor consists of a thin layer of porous silicon modified with Protein A. The system was probed with various fragments of an aqueous Human IgG analyte. The sensor operates by measurement of the Fabry-Perot fringes in the white light reflection spectrum from the porous silicon layer. Molecular binding is detected as a shift in wavelength of these fringes. IgG was added to and removed from the protein A-modified surface by changing solution pH in a flow cell, and the system was found to be reversible through several on-off cycles. The molecule used to link protein A to the porous Si surface incorporated bovine serum albumin (BSA). This approach was found to completely eliminate signal due to nonspecific binding. (paragraph adapted from ref. 13)

Porous silica materials made by low-temperature sol-gel process are promising host matrixes for encapsulation of biomolecules. To date, researchers have focused on sol-gel routes using alkoxides such as tetramethyl orthosilicate (TMOS) and tetraethyl orthosilicate (TEOS) for encapsulation of biomolecules. These routes lead to formation of alcohol as a byproduct that can have a detrimental effect on the activity of entrapped biomolecules.
Brinker and colleagues have developed a novel aqueous sol-gel process to encapsulate biological molecules (such as enzymes, antibodies, and cells) that uses neutral pH and room temperature and does not generate alcohol as a byproduct. Two enzymes widely used in biosensing applications, horseradish peroxidase and glucose-6-phosphate dehydrogenase, were used to prepare enzyme-doped silica monoliths and to investigate the effect of silica as host matrix on enzyme kinetics. The yield of the encapsulation process was close to 100% for both enzymes, and no significant leaching of enzyme was observed over time. Encapsulated enzymes followed Michaelis-Menten kinetics and maintained good catalytic activity. These novel enzyme-doped silica matrixes provide promising platforms for development of biosensors, affinity supports, and immobilized enzyme reactors. (paragraph adapted from ref. 14)

SUMMARY AND CONCLUSIONS

Much of the groundwork has been laid for bringing thin-film based biosensors to the marketplace. Biosensors based on monolayers, bilayers, organic polymer, and inorganic polymers have been described. Selectivity is intrinsic to most reported studies, and viable schemes, based on cooperativity or catalysis, have been described for boosting sensitivity. Much current effort is being placed on building biocompatible thin film materials in which the recognition element is active and accessible to the substrate. An additional important focus is development of surfaces that are sufficiently biocompatible that they resist biofouling and nonspecific binding. Perhaps these are the two most underdeveloped and least understood issues relating to biosensor development.

It is interesting to consider why more biosensors aren’t brought to market. One important reason is that big companies aren’t willing to take much risk unless there is a huge potential market (HIV and glucose for example). Thus, advancement will require start-up companies, probably involving university professors and university intellectual property. Unfortunately, there is at present no good mechanism for an academic to start a company. The SBIR and STTR programs are nearly irrelevant for this purpose, although they can be useful after a company is started. What is really needed are larger one-time grants ($250-$500K for 6-12 mos) that would get small companies off the ground and ready to apply for SBIR grants and private capital. There are government-sponsored programs in Germany that provide loans of about this size, that have to be paid off within a specified period of time: the amount that has to be paid back depends on the level of success of the company. Perhaps this is also a viable approach for bringing more small companies along.

Most university technology transfer offices are inept and most universities do not provide funds for patent costs. This means that much biosensor intellectual property is not protected, and this is a serious problem for any company that is considering using such technology. There should be a way for academics to protect intellectual property without having to rely on the largess of their university.

Academics are generally interested in “molecular recognition”, but industry is interested in robustness and market size. However, industry often harvests their ideas from published work by academics. This means that industry is trying to engineer systems that were never designed for the marketplace. This is another strong argument for government to support of those who wish to commercialize their biosensor technologies. Obviously, if an academic sees commercialization as a viable end-goal, then s/he will be thinking about robustness issues during critical early-design stages of project development.

There is no question that terrorist threats from abroad and within the U.S. will drive government to provide funds for biosensor research. I believe an even more serious biothreat comes from under-regulated and irresponsible domestic food producers, ever increasing centralization of food sources, those who pollute drinking water with biotoxins to reduce costs, and continued rapid growth in the U.S. population. Thin film-based biosensors of various sorts will play an important role in containing these threats, as will the many other technologies that are available for this purpose. However, some kind of integration of basic research with those who produce end-products is essential. Currently there is no viable mechanism for this kind of technology transfer, and thus the outlook for viable biosensing strategies in the next five years is rather bleak.
REFERENCES
