Biosensors: Blockbuster or Bomb?

Electrochemical Biosensors for Diabetes Monitoring

by Lance S. Kuhn

Convenience is an expectation of life these days, from bank machines to cellular phones to fast food. The medical world is not immune to this drive. There is a continual effort to push testing to the lowest level of professional skill possible, in order to allow patients easy access to the information they need to live their lives more fully. The ultimate in this battle is to make systems that patients can use by themselves, at home.

Electrochemical sensors have been a significant part of the move toward convenience and ease of use. For many years, sensors have helped reduce labor-intensive tests to simple one-step analysis, or even to hand-held home-use devices.

The earliest tests to become sensor-based were potentiometric ion-selective electrodes (ISEs), such as for pH, K^+, Na^+, and Cl^- and gas-sensing electrodes, such as for O_2 and CO_2. The use of these sensors is now standard procedure. Later electrochemical sensors included conductance tests for hematocrit (red blood cell volume), and then enzyme-based methods.

In 1956, Leland Clark stimulated the electrochemical biosensor endeavor when he described a method for making oxygen sensors that could be combined with enzymatic systems (the "bio-" part) to measure a whole new array of analytes.[1] The test he started with, and the one that is still the most important enzymatic sensor in the market, is for glucose. His method was patented in 1965, and applied to the Yellow Springs Instrument analyzer first sold in the mid-1970s. In this test, glucose oxidase oxidizes glucose in the presence of oxygen, turning the oxygen over as peroxide, which is then oxidized at the working electrode of an electrochemical cell. Following Clark's lead, others have taken this technology and applied it to lactate, creatinine, cholesterol, and other analytes of medical importance.

It took more than a decade longer before such a system was reduced to a hand-held instrument, reaching the ultimate level of convenience for the patient. In 1987, MediSense marketed the Exactech glucose sensor and, although it never gained a majority share of the market, it did generate a movement toward electrochemical sensors within the medical diagnostics community. In 1989, Eli Lilly began to market the Direct 30/30, a reusable biosensor that promised to revolutionize the home glucose monitoring market. Unfortunately, the user interface was not robust enough for the market, and this system was unsuccessful. Others have now followed, learning from the first systems, and three of the four largest self-blood glucose monitor (SBGM) makers have significant electrochemical sensor-based systems.

More recently, I-Stat introduced a hand-held system that has cartridges of up to six clinical tests at a time, with a total of 18 tests, all with electrochemical sensors. Wampole introduced a hand-held instrument that measures hematocrit by conductance. Next-generation sensor-based systems are beginning to emerge, including systems from Via Medical and TheraSense.

Based on this history, one might think that electrochemical sensors have taken the world by storm. Although there have been isolated successes, the market has not grown to the levels predicted ten years ago. Even in the glucose test market, easily the largest for electrochemical sensors, it took many years to accept them as a standard. Why is this true?

The Problem

Diabetes is a world-wide public health problem. When the body no longer produces insulin, or has developed a tolerance to it, and does not properly convert glucose into energy, diabetes is the result. Approximately 16 million Americans have diabetes; one third or more have yet to be diagnosed. Worldwide, these numbers are even more staggering. A 1994 World Health Organization report estimated that there are at least 110 million diabetics, and this number is expected to more than double in the next 30 years.[2]

FIG. 1. The Accu-Chek™ Comfort Curve™ biosensor test strip in the Accu-Chek™ Complete™ test device.

The complications of battling this life-changing disease are numerous: adults with diabetes have heart disease death rates two to four times those without diabetes; 60-65% of diabetics have high blood pressure; end-stage renal (kidney) disease is common among the diabetic population; retinopathy causes loss of sight; and the list goes on.

Much of the burden of this disease can be reduced or eliminated by early detection and improved self-care. The Diabetes Control and Complications trial[3] (DCCT), a 10-year nationwide study of 1,441 diabetics, conclusively demonstrated that good control of blood sugar delayed or prevented many of these complications at rates of at least 50% better than poorly-controlled subjects. Important to this good control is frequent, consistent, and accurate self-testing of blood glucose.

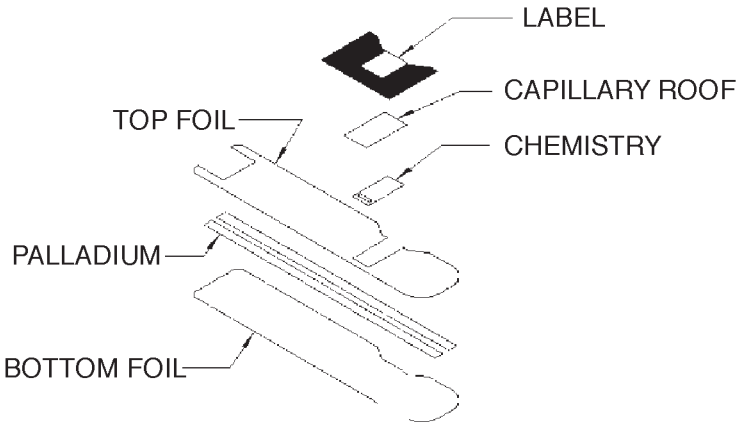

FIG. 2. An exploded view of the Accu-Chek™ Comfort Curve™ biosensor test strip.

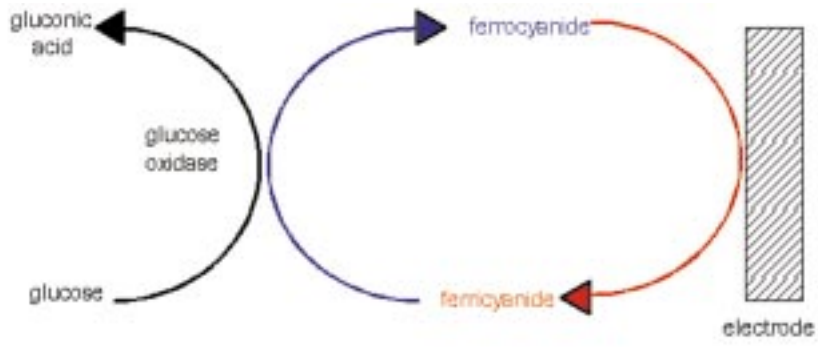

FIG. 3. The reaction sequence for glucose measurement on the Accu-Chek Advantage sensor.

Hurdles

The first hurdle any test in medical diagnostics faces is the need for specificity; it is no different for biosensors. A test is only as good as its ability to separate the signal due to the analyte of interest from another signal. In medical tests this is typically done with a biological specifier, such as an enzyme or an antibody. For glucose this specifier was defined long ago to be the enzyme glucose oxidase; more recently, glucose dehydrogenase has been used successfully.

The use of a biological molecule as a specifier brings an inherent second hurdle with it: instability. Large biological molecules are typically not stable outside the environment for which they were designed. The use of them in a test, especially in a test where they must be dried and stored for months or years, then used in extreme environments of temperature and humidity, requires considerable work, and often is not successful at all.

A third common hurdle for medical tests is sensitivity, as many molecules of interest in the body are at concentration of 10^{-6} M and below. This is not only a difficulty in absolute sensitivity, however, but is convoluted with specificity, in that many other compounds that might produce a competing signal are at similar, or even higher, concentrations. Fortunately, this is not the case for glucose, which is at millimolar concentrations in blood.

Market View

The slow acceptance of electrochemical sensors in the SBGM market was probably due to several effects unrelated to detection of the analyte molecule. First, the market for glucose biosensors, i.e., the diabetic population and their physicians, has changed drastically in the past 15 years. The hand-held optical instruments first introduced 15 years ago have evolved into very sophisticated instruments, capable of accurate and precise readings with very little effort from the patient. Any new tests, biosensor or otherwise, must meet or exceed these performance standards, which are based upon a calorimetric chemistry that has been under development for decades. Secondly, the manufacture of the electrochemical strips with the required electrode tolerances has proven to be both more difficult and more expensive than expected. Finally, the market has matured to a point where it is difficult for small players to compete, and necessary for the very large to be highly cost-conscious.

We will describe our efforts to develop a marketable biosensor, shown in Fig. 1, that is state-of-the-art, novel, inexpensive, and high-performing. A large part of our success is due to our ability to use two identical electrodes, and to allow the chemistry of the strip to control our glucose measurement. Using this biamperometric approach simplifies the cell requirements for the sensor. The product's life cycle will be followed to show how biosensors can make a difference in medical diagnostics. We will also peer briefly into the future of biosensors in this and other medical markets. Many excellent reviews delve deeper into biosensor technology; one recent paper broadly reviews the subject.[4]

The Device

There are several keys to making a competitive biosensor for the medical devices marketplace. Since this is a near-commodity market, cost (to the manufacturer) and price (to the consumer) of the individual sensor are major issues. As these devices are medical devices, which are used to diagnose potentially life-threatening incidents every day, they must be of very high quality, and the information displayed to the user must be accurate. The sensors must be easily manipulated by sight-impaired users, and the system must be very user-friendly to encourage more frequent testing for better control. In today's glucose testing market, features differentiate products in the marketplace; the ability of the system to interface with the physician's work, and software that allows users to track their results and regimen changes, are of growing importance.

In our sensor, shown in an exploded view in Fig. 2, two identical electrodes are used for amperometric detection, with no need for either a reference or a counter electrode of different size or material. The sensor reagent includes three active ingredients and several support ingredients. The active ingredients in most sensors of this type are a glucose-specific agent, an electron shuttle or mediator, and stabilizing agents for the glucose specifier to ensure long shelf life. The glucose specifier has typically been glucose oxidase or glucose dehydrogenase,

although the hexokinase reaction has also been used. The electron shuttle is often a ferrocene or ferricyanide derivative. In our case, glucose oxidase was used in the original Accu-Chek™ Advantage® product in 1994. Then, following a continuous improvement path, a glucose dehydrogenase-based sensor was introduced two years later. Potassium ferricyanide has been the mediator in all cases.

The mechanism of action of the sensor is shown in Fig. 3. The glucose in a sample reacts with glucose oxidase to make gluconic acid and the reduced form of glucose oxidase. The reduced glucose oxidase then reacts with ferricyanide to make ferrocyanide. The working electrode, poised at a potential positive of the rest potential of the mediator, oxidizes all ferrocyanide as it diffuses to the electrode. This generates a current directly proportional to the concentration of glucose in the solution. It also allows for a catalytic cycle of regeneration of the two key reagent constituents.

Sensor reagent improvements highlight a key to the marketability of this type of product. In a glucose oxidase-based test, the first interferent in the system is oxygen, which is the natural substrate for the enzyme. We are attempting to replace oxygen with potassium ferricyanide as an electron acceptor. The ferricyanide is not as efficient at shuttling electrons with the enzyme as oxygen; therefore, any oxygen in the solution can compete effectively for the enzyme site, producing a signal that is related to glucose, but not in the same way as the ferricyanide-mediated signal. Oxygen can give a positive bias in such a system, meaning measurements are skewed higher, limiting the accuracy at low glucose values.

Additionally, biological specimens contain widely varying oxygen levels. The oxygen partial pressure, pO_2, (a measure of dissolved oxygen content) of an average venous blood sample is about 40 mm Hg. This corresponds to a dissolved oxygen level of approximately 0.06 mmoles/L. For an arterial sample, one would expect much larger oxygen levels, with pO_2 reaching as high as 110 mm Hg, or 0.15 mmoles/L dissolved oxygen. Capillary samples typically have oxygen levels a little lower than arterial samples. Therefore, if one were to make measurements of glucose in the three different sample matrices, a glucose oxidase-based sensor could give three different results. Due to the seriousness of this problem, any differences

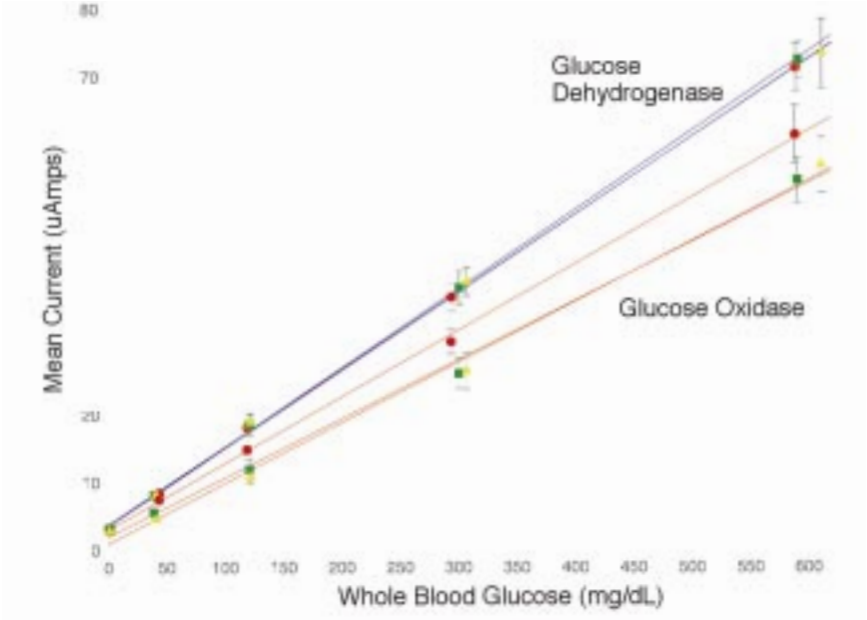

FIG. 4. A comparison of the effect of oxygen on the glucose test result, for a glucose-oxidase-based system (red regression lines) versus a glucose dehydrogenase-based system (blue lines). Three levels of oxygen are shown: a standard venous level of 21.7 mm Hg (red circles), a standard capillary level of 94.3 mm Hg (green squares), and a hyper-oxygenated sample at 182.8 mm Hg (yellow triangles). The five glucose levels were tested at n=12, and error bars represent ± two standard deviations from the mean.

in samples must be described in the product literature, and claims must be approved by the Food and Drug Administration (FDA) for use in such settings. This has led to few systems being approved for use for all samples.

The route to an improved sensor that can be used in all medical situations and, more importantly, gives unvarying results in these wide-ranging circumstances was to replace the glucose oxidase in the strip with glucose dehydrogenase. Glucose dehydrogenase does not interact with oxygen, and therefore is unaffected by variable oxygen concentrations in the sample. Fig. 4 shows the reduction in oxygen dependence afforded by such a change. Note that there is a > 10% difference in calibration slope for venous versus capillary data in the oxidase system. In the dehydrogenase system, the calibration slopes are essentially superimposable. Similar results have been shown in a clinical setting.[5]

Other concerns for a successful business venture in biosensors are cost and complexity of manufacturing. Biosensors, by definition, include biological components; and as they must make an accurate measurement in some of the worst samples, there is a tendency to create a rather complex system that becomes too difficult to manufacture. To date, the sensor reagent mixture remains complex, for both biological (enzyme degradation) and manufacturability reasons. However, the electrochemical cell does not necessarily have to be complex.

In a typical electrochemistry system, a reference electrode is used to standardize the potential of the working electrode to a known value. This allows one to know, very accurately, at what potential the working electrode is poised, and to assure that that potential is both sufficient for linear current generation and low enough to avoid as many biological oxidations as possible. But it complicates the manufacture of the disposable strip, as it takes extra steps to create a reference electrode. In the case of the Advantage® strip, we have eliminated many of these difficulties by using two noble metal electrodes that are exactly the same.

A depiction of the Accu-Chek™ Comfort Curve™ strip is shown in Fig. 2. Note the simplified architecture with the similar electrode configuration. Essentially, two identical palladium electrodes are sealed between two thin sheets of plastic, with cut-outs for electrode contact and chemistry/sample interface. Palladium was used in this sensor because of its relatively low cost for sputtered films (at that time, much lower than for gold) and its resistance to surface oxidation. A small volume (6 μL in the configuration shown) of the sensor reagent is dispensed and dried in the open window of the strip. Data is collected using chronoamperometry; after a short reaction period, a potential difference is applied between the elec-

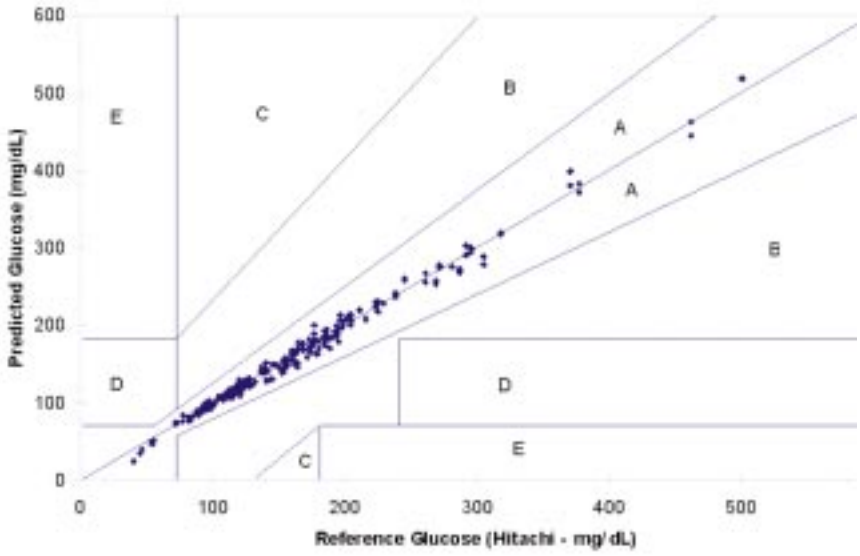

FIG. 5. A standard Clark Error Grid analysis of the calibration curve assignment for an Advantage® biosensor product. The lettered zones represent clinically relevant regions of performance: A: clinically accurate readings; B: results that would lead to benign action or inaction by the user; C: results that would lead to unnecessary corrections; D: results that would lead to inaction when action is necessary; E: results that would lead to treatment opposite of what clinical accuracy would call for.

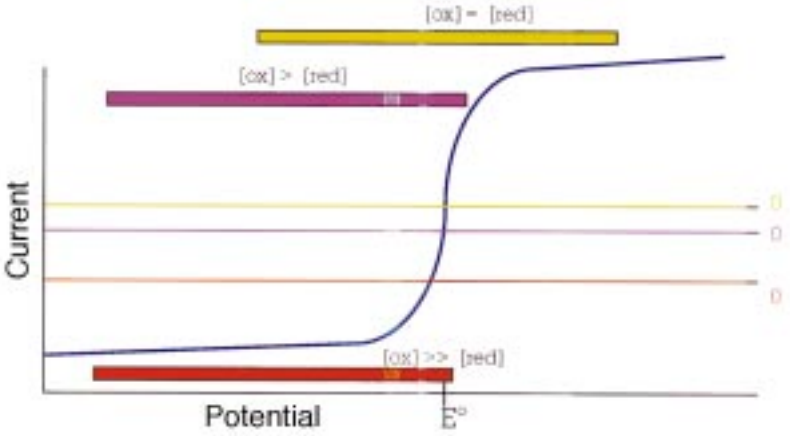

FIG. 6. The general principle of the electrochemical mechanism of Advantage® sensors is illustrated. The yellow bar shows the 150 millivolt potential window with equal oxidized and reduced mediator concentrations (a situation we must never reach in the sensor); the purple bar shows the potential window with slightly more oxidized than reduced mediator (high glucose concentrations), and the red bar shows the situation at oxidized >> reduced (low glucose.)

trodes and current collected for a specified time. Fig. 5 demonstrates a typical calibration curve for a set of about 300 measurements using capillary blood samples. Precision, accuracy, and linearity are excellent.

Of course, there is no free lunch, and we pay for this by a slightly more complicated or less well-defined electrochemical mechanism. Our sensor works through a combination of very basic electrochemical principles, though in an unusual way.

The Nernst equation,

$$E = E^\circ + \frac{0.059}{n}\left(\log \frac{[ox]}{[red]}\right)$$

applies to equilibrium, i.e., zero net current. This is the case, for instance, if one allows a reaction to proceed to equilibrium, then measures the energy level of the system, i.e., the open circuit potential. Such a case is considered here, where we always begin with the same ratio of [ox]/[red] (approx. 0.05% ferrocyanide in 99.95% ferricyanide). The glucose-specific reaction described earlier converts a fixed amount of the ferricyanide to ferrocyanide under a condition of open circuit. Thus, if we allow the reaction to proceed, different glucose concentrations will give us different [ferricyanide]/[ferrocyanide] ratios and, therefore, different open circuit solution potentials.

In the case of our sensor, the two electrodes of the electrochemical cell are identical. One way we can create a difference is to apply a potential difference between the two, changing the energy levels such that current must flow. If that potential difference is large enough (150 mV), diffusion-limited (Cottrellian) current can occur at each electrode.

The limiting current will be obtained at the electrode at which the limiting reaction is occurring; for instance, if [ox]>>[red], the oxidation of [red] to [ox] will be the limiting reaction (and in this case, the one we wish to measure at the working electrode). The two electrodes must quickly come to agreement, with a separation of 150 mV and with currents equal in magnitude. Because the working electrode reaction generates a small amount of current, the potential of the other electrode (the counter electrode) will position itself just far enough negative to give an equal cathodic current to the anodic current measured at the working electrode, as shown in Fig. 6. The working electrode will then poise itself as far positive as necessary to maintain the 150 mV potential difference. This should always be in a region of purely diffusion-limited current generation. According to the Nernst equation, the actual potential of the working electrode will be more negative as the concentration of glucose (and, therefore, ferrocyanide) is increased.

We need not concern ourselves with the true (referenced) potential. Different mediators will have different rest potentials and, therefore, actual electrode potentials will vary depending on the chemical system. A mediator should be chosen with as low E° as possible, so that the oxidation of extraneous compounds, e.g., uric acid or ascorbic acid is limited. However, regardless of the mediator used, very high redox indicator concentrations must be used, such that [ox] is always greater than [red] and the limiting current is always generated at the working electrode. The use of a high concentration of a redox mediator allows control of the actual potentials of the identical working and counter electrodes by the glucose reaction. As long as the initial mediator concentration is greater than two times the stoichiometric concentration of sample analyte, one maintains analyte-limited currents. In the case of potassium ferricyanide, the high concentrations of mediator do not create a problem, since this com-

pound is highly soluble, inexpensive, and easy to obtain. If too low a concentration of the oxidized mediator in the strip was used, the signal due to glucose would peak, then diminish, even as glucose increased, because the limiting reaction would become the reduction of the oxidized form of the mediator at the counter electrode. This would result in a very dangerous test system for the patient. Great care has been taken to insure that this will not be the case, loading the sensor reagent with several times the amount of mediator necessary to meet even very high glucose levels.

Now the steps to address the keys to making a competitive biosensor for the medical device market have been taken. The cost of the sensor has been reduced by eliminating one of the complicating features of an electrochemical sensor, the reference electrode. By developing the reagent according to the chemical and electrochemical principles this simple test has been made very accurate and reproducible. The electrochemical sensor eliminates some of the drawbacks of an optical test system, such as stray light interference and contamination of the meter by the sample. The strip is large enough to be easily manipulated, has a colored sample touch pad, and the end nearest the patient can be handled without fear of touching the sample or adulterating the test. The resulting device is an electrochemical sensor system with a high degree of linearity, excellent accuracy and precision (Coefficient of Variation < 3%) across the clinically-relevant range of glucose concentration. Of course, there are still improvements that can be made.

Future Directions for Electrochemical Biosensors

The newest sensor in the Advantage® line, the Accu-Chek™ Comfort Curve™ in FIGS. 1 and 2, takes advantage of capillary fill and an improved reagent to test with even smaller sample volumes and more accurate results. The former is important to patients who prick their finger several times daily, because smaller sample volumes necessarily mean finger punctures that are less severe. More accurate, and especially more robust, systems are always at the top of our priority list as we try to provide the customer with useful information.

A look into the future shows a significant shift in the way glucose biosensors are used.[6-8] The results from the DCCT, and several studies since then, clearly demonstrate that diabetics who closely monitor their glucose, and act according to the test results, have better long-term outcomes. The obvious way to make this possible for our customers would be to provide them with a sensing system that continually, or nearly continually, displays accurate glucose readings, which are generated from as minimally invasive a system as possible. The "holy grail" of this work is the noninvasive systems that allow testing with no finger sticking and nothing in the body. However, these systems appear still to be far away.

On the other hand, many groups have reported methods for minimally invasive glucose measurement on a relatively continuous basis. Among these are many electrochemical methods. One, the VIA Medical probe, is a venous catheter system that constantly measures glucose in the blood, and is already on the market for hospital use.

A few others, further from the market, are worthy of note. Wilson and coworkers at the University of Kansas and in Europe, have been pursuing the implantable glucose sensor for many years.[9-11] Their sensor uses glucose oxidase immobilized at the surface of a working/reference electrode combination. Here, no mediator is employed, and the sensor relies on the generation of peroxide from the native oxygen reaction; the peroxide is then oxidized at the working electrode. Polymer layers surround the chemistry to protect it from the body, and the body from the chemistry. This sensor is used subcutaneously to measure glucose on a continuous basis. Similar sensors have been reported by other research groups, some relying on the direct measurement of oxygen consumption, and others on the oxidation of the peroxide produced in the enzymatic reaction.

MiniMed has recently described a similar system to the FDA in an attempt to gain approval for use in hospitals and some patients.[12] The results of FDA's review of their data will have a dramatic effect on research in many companies and research laboratories around the world.

Heller and associates[13-15] have taken a different approach. In their sensor, a wired enzyme/mediator combination is stated to reduce oxygen dependency of the sensor, and to provide a reliable result continuously. They attach glucose oxidase or glucose dehydrogenase to a poly(vinylimidazole) polymer backbone, and attach a redox mediator (eg., $Os(bpy)_2Cl$) to another part of the polymer. These two molecules are then relatively free to interact, exchanging electrons which then travel "through" the polymer "wire" and lead to a final signal from the mediator at the electrode.

Even more fascinating, but also more difficult, are electrochemical methods to measure glucose without the aid of biological specifiers. Such methods would allow reduced biological reaction to the testing device cycle, and less-complicated sensors. Several groups have used pulsed AC and cyclic voltametric methods to measure the direct oxidation of glucose at a catalytic electrode surface such as platinum or metal oxides.

Microfabrication techniques are leading to the proliferation of microsensing devices, which will lead biomedical sensors into entirely new fields and allow for arrays of tests on single small devices. One example of such research is at Duke University, where the research groups of Buck and others have developed arrays of tiny electrodes that monitor heart electrical activity and important clinical parameters.[16] Another use of microfabrication showed 400 individually-addressable microelectrodes on a single 1 cm^2 chip, allowing spatial resolution of analyte distribution in a small area.[17]

These are only a few of the vast array of research efforts currently exploring biomedical, especially glucose, sensors. All these efforts will eventually face the same issues as those we have encountered with first generation sensors. In addition, they will face the difficult tasks of making a measurement in the very harsh environment of the body, and making these tests very stable and reproducible. Their success in passing these tests will bring significant changes in near-patient testing of many medical and biological compounds. ■

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