Immunosensors: The next generation

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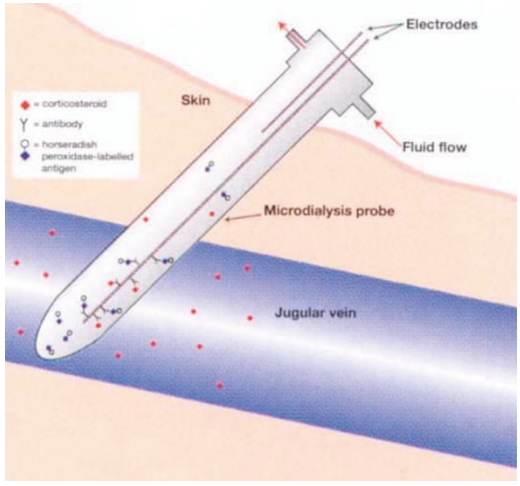
The development of the radioimmunoassay by Yallow and Berson in 1959[1] revolutionized clinical analysis. Since then, much ingenuity has been expended to convert this laboratory method into safer, easy-to-use formats that can be more broadly applied. Success in this respect is probably best exemplified by over-the-counter pregnancy tests using colored latex particles to visualize antibody binding human chorionic gonadotropin (HCG), which are currently used by millions of women around the world. Increasing interest has also focused on the development of immunosensors—devices in which the diagnostic immunochemical reaction is coupled to a transducer. In this issue (pp. 466–471), Christian Cook[2] presents an interesting format for an immunosensor, combining an immunoassay, an electrochemical sensor and a microdialysis membrane to create a probe that can be implanted in living tissue to track hormonal change over time.

Harnessing the power of affinity reactions for real-time and on-line diagnostics has been one of the principal driving forces of biosensor research for nearly two decades. In 1982, Janata and Blackburn[3] gave us the concept of the immunoFET, elegantly combining the worlds of electronics and immunoassay with a transistor effected by a biological reaction. This idea proved to be not only ahead of its time, but ahead of the materials technology necessary to put it into practice. The mass change resulting from affinity reactions occurring at the surface of piezoelectric devices[4] was another bright idea that initially fell foul of the physics associated with biological fluids, although recent advances have led to compelling demonstrations of such devices.

A significant breakthrough came with the introduction of real-time biospecific assays based on evanescent-field technology. Seminal work by Lundströms's group[5] on surface plasmon resonance based affinity sensors was translated by Pharmacia (Uppsala, Sweden) into commercial success in the early 1990s with the launch of the BIAcore analyzer. This machine facilitated, for the first time, widespread monitoring of antibody–antigen reactions in real time, thus revealing a plethora of new data beyond the simple concentration information available with conventional assays.

Although proving invaluable in, for example, pharmaceutical research applications, this generation of optical instrumentation remains relatively cumbersome and expensive. Electrochemical devices for home blood glucose measurement have shown, however, how clinically acceptable data can be generated with inexpensive pocket-sized instruments suitable for over-the-counter sales[6]. Electrochemistry can also be applied to immunoassay, which when performed in extremely low volumes can yield ultrasensitive assays with detection limits in the order of 10^{-21} moles[7].



Immunosensor combining an immunoassay, electrochemical detector and microdialysis membrane, as described by Cook.[2]

The demand for clinical techniques that can provide measurements more cheaply and preferably without the need to collect and/or transport samples has provided impetus to research on continuous in vivo monitoring of key analytes—in particular metabolite monitoring, which is driven by the huge need of diabetics and applications in critical care[8]. Microdialysis, where the sensor or assay is separated from the sample by a dialysis membrane incorporated into a probe, has proved invaluable in obtaining near real-time research data on brain function and metabolism in living animals, and it may prove useful for clinical use in certain critical situations.

In the present paper, Cook has chosen corticosteroids as a model analyte because they are present at relatively high levels in the blood and exhibit large circadian changes and fluctuations in response to stress. His sensor consists of polyclonal antibodies to corticosterone immobilized on a platinum electrode housed inside a needle-shaped microdialysis probe. The probe can be implanted intravascularly in animals by a simple catheterization process. Following equilibration with the blood, a solution containing corticosteroid conjugated to horseradish peroxidase is introduced into the internal cavity of the probe. The labeled analyte competes with the endogenous hormone for binding sites on the electrode and, following an in situ washing step, can be assayed by amperometric detection of the enzyme activity. The probe is regenerated by flushing with 1 mM HCl. Using this protocol, measurements can be made every 3 minutes. Cook has therefore shown that in vivo microdialysis can be extended from metabolite monitoring to semi-continuous measurement of antigens such as hormones.

There is little justification for continuous clinical monitoring, unless the analyte varies rapidly and unpredictably, and there is a suitable therapy available[7]. Research applications of in vivo monitoring, however, abound and one of the most flexible approaches to a range of potentially interesting analytes is immunoassay. The paper by Cook thus describes a very welcome addition to the next generation of analytical tools.

If sufficient sensitivity can be achieved with a stable in vivo probe, considerable scope exists for a wide range of research applications using specific antibodies for various analytes, including a range of hormones, protein markers, allergens, infectious agents, and toxins. It is clear that the next commercial battles in the burgeoning biosensor field with be fought over immunosensors. In vivo monitoring is an important long-term objective, especially with a view to understanding the effects of drug delivery at the cellular level. In the shorter term, however, the lucrative in vitro diagnostic market is more likely to occupy companies' time, with the search for a hand-held immunosensor for intermittent use.

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