Novel materials promise faster and simpler biosensors for gene detection

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Electrically conducting plastics and nanoparticles can aid the simultaneous measurement of the activity of many genes, advancing basic research and medicine.

Monitoring gene expression—the process of turning on specific segments of chromosomes—provides crucial progress in biomedical research, clinical diagnosis, forensic science, and an increasing number of related disciplines. The so-called transcription of genes produces messenger RNA (mRNA) that is then converted to proteins, the end products of gene expression that determine the functions of cells and tissues. The human and other genome projects have provided rich databases of genes, often of unknown functions. To begin to understand how organisms work, it is important to be able to first measure and quantify the presence of specific mRNA sequences that indicate that a gene is present and turned on.

Gene microarray technology holds special promise because it simultaneously measures the expression of many gene fragments. Current gene-detection technologies, however, still require extended and expensive procedures to prepare samples. Therefore, any improvements in speed and simplicity should boost the potential of microarray and related technologies. Such advances will also make rapid genetic analysis accessible for clinical and forensic applications.

A promising new platform for cheap and rapid biosensors arrived with the discovery of conducting polymers (CPs), electrically conducting plastics that can be effectively functionalized with biomolecules. By attaching or trapping short, synthetic-DNA fragments—oligodeoxynucleotides (ODNs)—in the polymer, the resulting sensor film responds to the presence of complementary sequences in the sample.

Conventionally, the detection of DNA is achieved by tagging the sample with a fluorescent or radioactive tag. Both of these tagging methods, however, suffer from shortcomings, including limited chemical-tagging efficiency and hazardous-waste disposal. CPs, on the other hand, can be used as active substrates to directly report the biological recognition event: DNA hybridization in this case. This becomes possible as a result of changes in the electro-optical properties of the polymer, which are induced by sample hybridization. As a result, these sensors are able to rapidly report the presence of specific gene fragments within an unlabelled sample.

Constructing effective CP-based sensors depends on linking the DNA probes to the polymer film in a way that maximizes the readout signal upon hybridization. At the Polymer Electronics Research Centre at the University of Auckland, we have explored two approaches. The fastest and simplest route to a specific gene sensor involves trapping DNA fragments in a thin CP

Figure 1. Labelling DNA probes with nanoparticles increases the amplification in a gene sensor based on polypyrrole, which is a conducting polymer (CP). (Left panel) A layer of nanoparticle-labelled probe strands attaches to target oligodeoxynucleotides (ODNs) on the CP film after hybridization. (Right panel) Unlabelled ODN probes bind to the CP film. The bar graph shows the relative responses from both sensors in charge transfer resistance ($\Delta R_{ct}$).
Using polypyrrole, a well-studied CP, such sensors can be made within a matter of minutes by using electrically induced film deposition (electropolymerization) to obtain a film of precisely controlled thickness—typically 50–100nm—that contains the sample DNA. Following hybridization with probe strands, we can detect the presence of complementary strands by using impedance spectroscopy, a technique that measures interfacial characteristics of the sensor film. The impedance change following hybridization is most pronounced at frequencies that are characteristic of the charge-transfer resistance: a measurement of the ease with which charges can cross the interface between the electrolyte solution and the polymer film. The presence of double-stranded DNA—created when a single-stranded test fragment hybridizes with a trapped, single-stranded probe—increases charge-transfer resistance. This is likely due to both electrostatic effects and reduced access of ions from the solution.

In a second approach, covalent bonds attach ODNs to the polymer. This requires the synthesis of modified monomers that possess linker groups appropriate for binding to end-modified DNA-probe strands. While chemically more demanding than simple entrapment, this approach holds the promise of molecular tuning. That is, linker groups can be designed to maximize the transduction of the hybridization event to the polymer backbone. Recently, we started to systematically study the effect of linker properties on sensor performance and have achieved promising results. This approach also uses impedance spectroscopy to measure the resulting hybridization.

We are also exploring the use of other nanomaterials to further improve sensor properties. For example, our work shows that labeling probes with semiconductor nanoparticles increases sensor response as compared to using unlabeled-probe strands. This amplification results from the comparatively large size and charge of the employed nanoparticles and indicates the potential of new sensor designs that harvest the properties of both nanomaterials and CPs (see Figure 1).

As indicated by our and others’ work, CP-based electrochemical sensors—possibly in conjunction with other new nanomaterials—hold great promise to provide the next generation of affordable and rapid gene sensors. Nevertheless, this growing area of bionanotechnology faces a number of major challenges. First, the sensitivity cannot yet match the detection limits of fluorescent-labelling assays. Second, while the route to sensor miniaturization appears straightforward, much work is still required to realize large-scale CP-based microarrays. Finally, although we have some understanding about the mechanisms underlying the sensor response, more information is required to realize the full potential of this technology. To overcome the first challenge, we are currently investigating opto-electrical readout, which should boost the sensitivity.

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