Opinion

Biochip sensors for the rapid and sensitive detection of viral disease

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Abstract

Recent advances in DNA and protein microarray methodology and the emerging technology of cell-based sensors have massively increased the speed and sensitivity with which we can detect viral infections. The advantages of the multi-parameter microarray technologies could be combined with the speed and sensitivity of cell-based systems to give 'cell-omic' sensors.

In 2003, China took measures to contain an outbreak of 'flu-like illness' [1]; when the same disease (which came to be called severe acute respiratory syndrome, SARS) began to appear in other countries, the World Health Organization initiated a global response [2]. This incident highlighted, on a world stage, the need for rapid and accurate techniques for pathogen identification. Failure to have such tools puts lives at risk by severely hampering containment and effective vaccination strategies.

Over the past few decades, the identification and characterization of infectious agents has been refined and improved, resulting in highly sensitive and precise methodologies that will soon be able to measure individual molecules. This sensitivity comes at a cost, however, in terms of time, complexity of assay, and robustness of measurements, and this can have a negative impact on patient care. The prognosis for the majority of serious infections is vastly improved by early intervention, so the development of rapid detection and identification methods is essential, but this must not come at the expense of sensitivity. In the case of hepatitis C infection, for example, diagnosis needs low levels of virus to be detected [3], and this demands a high level of assay sensitivity. For these reasons there is an ever-increasing requirement for rapid, sensitive technologies that provide better diagnosis and clinical management of infectious diseases. In an effort to address that need, modern medicine has seen a revolution in new high-throughput approaches. Advances in genomics, microarrays and imaging technologies, in particular, have revolutionized the way in which infectious-disease problems are being addressed. Here, we briefly examine how such technologies are being applied to the detection and identification of viruses and the impact such systems might have in the clinic.

DNA and protein microarray approaches

Until recently, virus detection and identification in the clinical setting has been centered around immunological or PCR-based techniques. One of the primary immunological techniques is the use of enzyme-linked immunosorbent assays (ELISAs) for the detection of circulating virus-specific antibodies. By contrast, reverse transcriptase (RT)-PCR is used to detect the presence of viral genomes or specific viral genes. A combined approach using both techniques overcomes detection problems when either the infection produces a weak antibody response or when virus-specific transcripts are in low abundance. Both these approaches have well documented limitations, however. Immunological tests are hampered by the need for specific antisera that are both laborious and time-consuming to produce, whereas PCR, while being a definite advance in sensitive virus detection, is prone to failure and false recordings and is limited in its ability to identify multiple

viruses simultaneously [4]. We therefore need a rapid, sensitive approach that is capable of identifying multiple viruses in parallel; this need is being addressed by the development of DNA and protein microarrays specifically designed for virus detection and identification.

The basic design principle is the same for all forms of microarray, whether based on DNA, protein or cells. Specific molecular 'targets' are detected simultaneously within the sample of interest by an array of 'probes'. The probes, often numbering thousands, are chemically attached in an array format to a solid substrate to construct either a DNA or a protein microarray (Figure 1). But the microarray concept is not limited to the use of just DNA or protein probes. Indeed, in recent years the concept has been greatly expanded to include the production of all manner of arrayed probes: cells, glycans and carbohydrates, to name but a few. The significance of the microarray to the field of infectious diseases is the parallel detection capabilities of the system (covered in more detail in [5]). Microarrays offer the ability to achieve simultaneous detection of many targets, and through optimization this can be achieved without detriment to sensitivity.

DNA microarrays for viral analysis can be divided into viral chips and host chips, and each can be applied not only to detection and identification but also to the monitoring of viral populations. In 1999, we and colleagues [6] described the first viral DNA microarray for the temporal profiling of viral (human cytomegalovirus, HCMV) gene expression. Treatment of infected cells with cycloheximide or ganciclovir was used to block *de novo* protein synthesis or viral replication, respectively, and the microarray was then used to generate expression profiles of the viral genes represented on the chip. Using this approach, HCMV genes were assigned to immediate-early, early or late expression classes, depending on their expression profile in response to the drug treatments. If the expression profile is sufficiently unique, it can be used as an identifying hybridization signature for the molecular staging of an infection.

We described the idea of unique hybridization patterns being used for the identification of viral inhibitors [6], and in 2002 this idea was applied by Wang *et al.* [4] to the detection and identification of viruses. The authors [4] described the use of viral DNA microarrays to produce hybridization signatures of viral sequences that effectively serve as 'viral barcodes' for the identification of known, related or novel viruses. By taking advantage of the highly conserved regions within gene families, the authors were able to produce an array that could identify related viruses and discriminate between serotypes. The ability to distinguish subtypes is critical to effective infection management in the clinic: variola virus, for example, is an orthopoxvirus that causes smallpox and has two subtypes, variola major and variola minor, of differing pathogenicity. Laassri *et al.* [7] addressed the problem

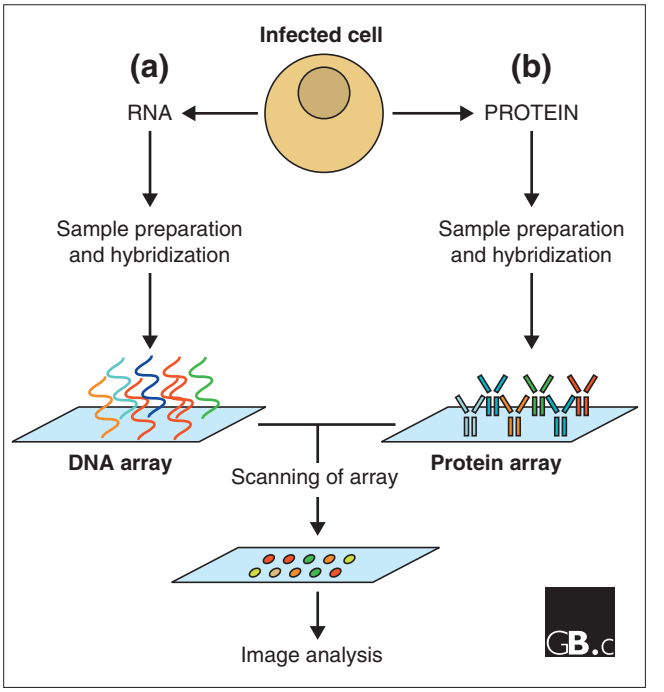

Figure 1
The structure of microarray experiments. **(a)** To obtain gene-expression profile data from a cDNA microarray, or chip, RNA is first extracted from an infected cell. The RNA is then reverse-transcribed and labeled ('sample preparation') and prepared RNA is hybridized to the chip. **(b)** Protein microarrays may have either antibodies or antigens arrayed as probes. Antibody probes can be used to detect antigens from an infected cell, and vice versa, following sample preparation and labeling. In both cases (a,b) the hybridized chip is scanned and the image processed to provide corresponding profiles.

of orthopoxvirus subtype discrimination by producing an array capable of correctly identifying four of the orthopoxvirus species. Similarly, arrays have been developed for the detection and distinction of hantaviruses [8] and are capable of distinguishing between isolates that have up to 90% sequence similarity. Other groups have focused on the genotyping of viruses such as human immunodeficiency virus (HIV) and influenza [9,10].

The ability to monitor the divergence of virus strains is critical for maintaining the effectiveness of current vaccines and ensuring the safety of vaccines that use live, attenuated viruses. Cherkasova *et al.* [11] have demonstrated the use of oligonucleotide microarrays in the analysis of vaccine-derived polioviruses. They describe two chip-based approaches. The 'microarrays for resequencing and sequence heterogeneity' (MARSH) method uses probes with overlapping sequences from within the coding region of the gene for the poliovirus structural polypeptide VP1 to detect point mutations that have occurred and to determine regions of differing genome stability. By contrast, the 'microarray analysis of viral recombination' (MAVR) method can detect recombination events

within virus strains by analyzing patterns of hybridization to probes unique to specific virus strains. Used in combination these chips provide a rapid genotype profile.

Viral chips provide a unique signature derived from the viral transcriptome or genome alone. An alternative approach is to examine the host response: changes in host gene expression provide a molecular signature of infection, an idea explored by Cummings and Relman [12]. The availability of commercial chips covering the whole host genome, from companies such as Affymetrix, allows genome-wide changes to be examined. Alternatively, smaller customized host chips can be constructed with a more restricted number of probes. One of the first groups to adopt this approach identified 258 cellular mRNAs whose level changed by a factor of four or greater before the onset of HCMV DNA replication [13]. Later, Domachowske *et al.* [14] examined pneumovirus strain differences and their ability to induce antiviral inflammation, and van't Wout *et al.* [15] examined HIV-1 infection in $CD4^+$ T cells to identify changes in host gene expression that were specific to HIV infection and that did not occur in cells that had been heat shocked, treated with interferon or infected with influenza A virus. Host gene signatures identified included pro-inflammatory genes and genes involved in endoplasmic-reticulum stress pathways, the cell cycle and apoptosis. A cardinal signature and common molecular thread for all infections appears to be the markers in the interferon pathway.

Microarray applications such as those described above offer an accurate, rapid and sensitive method for the detection and identification of viruses, but they have important limitations that should be considered. The production of robust unique hybridization signatures - viral barcodes - which can be used to correctly identify a viral infection depends on a number of influencing variables. For example, signatures may be altered dramatically according to variations in the viral load, the stage of infection or the tissues sampled. Obtaining the DNA for hybridization could also be problematic for some infections: infected tissues may be inaccessible and could yield little nucleic acid.

Protein arrays can also be constructed for the detection and identification of viruses. Viral antigens can be arrayed and used to detect serum antibodies, or antibodies can be arrayed and used to detect pathogens. Bacarese-Hamilton *et al.* [16] applied protein microarrays to the detection of antibodies to the protozoan parasite *Toxoplasma gondii*, rubella virus, CMV and herpes simplex viruses (HSVs) type 1 and 2. Antigens were arrayed and used to detect serum immunoglobins IgG and IgM down to 0.5 pg, and the system was validated by comparison with existing ELISAs. The results showed 80% agreement between ELISA and array, and confirmed that smaller reagent and sample volumes are used by the array. They also highlighted the advantage of the array's internal calibration curve: by processing the calibration curve on the array and not in separate tubes, as is done in ELISA, matrix effects that are known to bias ELISAs were reduced.

Once constructed, protein arrays can be air-dried and easily stored at room temperature [17]; their production and use are readily automated and they offer a cost-effective alternative to ELISAs. In contrast to DNA arrays, protein arrays cannot provide a readout of global changes in protein expression, since extensive libraries of globally expressed proteins simply do not exist [18]. It is still possible, however, to generate protein analyte 'signatures' by using a specific selection of targeted proteins. For instance, cytokine responses to viral infection can vary greatly between viruses; by arraying antibodies to a spectrum of cytokines it is possible to generate a 'cytokine signature' of infection that is readily identifiable. The application of such technology to the clinic would, however, require a concerted effort to characterize and collate such cytokine signatures. Considering that each signature is subject to a number of variables, each of which can produce a significantly different output, establishing a catalog of viral identifiers that are consistently accurate would be no mean feat.

Cell-based detection

Despite the rapidity and sensitivity offered by systems that use microarray detection and identification, recent work has demonstrated that it is possible to engineer cell-based systems that outstrip microarrays in terms of speed of detection. Rider *et al.* [19] demonstrated the use of engineered B cells capable of detecting pathogens within 3 minutes (Figure 2). Their CANARY sensor (cellular analysis and notification of antigen risks and yields) comprises B cells that

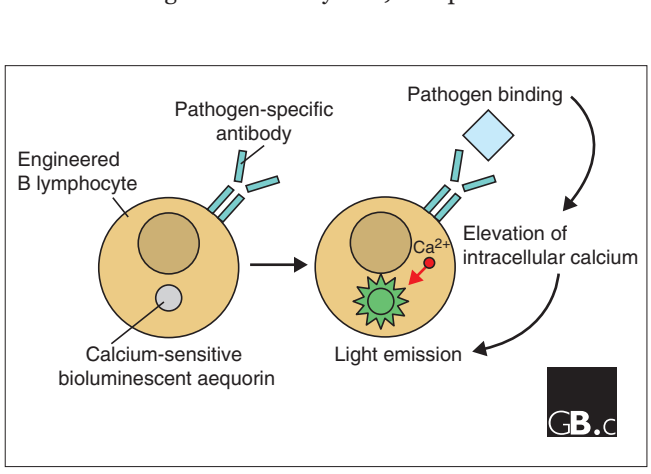

Figure 2
The CANARY method (cellular analysis and notification of antigen risks and yields) [19]. B lymphocytes were engineered to express calcium-dependent bioluminescent aequorin in the cytosol as well as pathogen-specific antibodies on the cell surface. Ligation of the antibody by the pathogen causes an elevation in intracellular calcium ions, thus triggering emission of light from the aequorin within seconds of pathogen contact.

express the calcium-dependent bioluminescent protein aequorin together with membrane-bound pathogen-specific antibodies. Binding of the pathogen to the cell-surface antibodies triggers an elevation in intracellular calcium ions that in turn causes the aequorin to emit light, all within a matter of seconds. One of the engineered B-cell lines described by the authors could detect spores of *Bacillus anthracis*, a pathogen already feared by the general public in relation to terror attacks, highlighting the relevance of such a technique. These investigators [19] used naturally occurring pattern-recognition molecules - antibodies - but such systems are by no means restricted to the natural world; for instance, Golumbfskie *et al.* [20] have investigated the possibility of developing synthetic systems capable of specific recognition between polymers and surfaces. Such biomimetic approaches may have future applications in both cell-based and microarray sensor technologies.

More recently, Perlman *et al.* [21], have described multidimensional drug profiling by automated microscopy. The authors used automated microscopy to create profiles, analogous to those generated by microarray data, of changes in cellular phenotype resulting from drug treatment; the profiles could then be used to categorize various unknown drugs [21]. This cell-array technique should complement existing technology and allow rapid, cost-effective collection of data or individual cellular responses. Although the results are in the context of drug treatments, one can easily imagine the application of such a technique to the detection of infectious diseases. A system such as this, capable of creating profiles based on phenotypic changes in individual cells, would be a powerful tool. This technology illustrates how the ability to measure changes at the molecular level can allow us to turn individual cells into sensors.

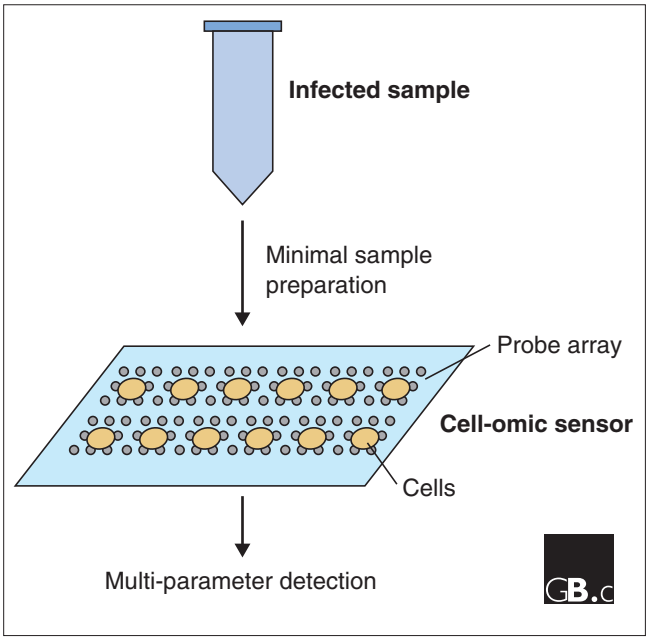

Figure 3
The concept of 'cell-omic sensors'. Cell-based detection systems can be combined with arrayed probes to allow multi-parameter analysis. By arraying cells in a monolayer on top of probes (such as antibodies), it would be possible to detect changes in multiple cellular components simultaneously. Components secreted from the cell or expressed on its surface could be detected directly by the probes; detection of intracellular components would require the use of more sophisticated techniques. Information would be collected directly from the underlying probes through detection systems positioned below the probes.

'Cell-omic' sensors

Despite the fact that microarray-based technologies are becoming increasingly rapid, cheap and ever more sensitive, there are still drawbacks. One approach to maximizing the effectiveness of existing technology is to combine complementary technologies (Figure 3). Microarrays provide the opportunity to develop a system whereby multiple viral infections can be identified in parallel by their hybridization signatures or 'viral barcodes'. Cellular systems, such as the light-emitting B cells engineered by the Rider group [19], while individually not having the parallel capabilities of the array, provide an extremely rapid detection system. In the future, therefore, we could see the production of hybrid technologies: 'cell-omic sensors', which have the parallel high-throughput capabilities of arrays coupled with the speed of the engineered B cells. This may take the form of microarrays constructed by arraying a panel of engineered cells, for instance, or even synthetic biomimetic systems. Alternatively, advances could allow arrays to be constructed that combine cellular sensors with gene or protein probes. It may be possible to take advantage of the immune system's natural pathogen sensors - macrophages or dendritic cells for example - arrayed onto protein probes or sensors of some description in such a way that cellular changes induced by contact with a pathogen can be measured in real time.

The goal of generating hybrid arrays of cells within arrays of protein probes is increasingly feasible with advances in science and technology. Ultimately it might be possible to engineer a 'microarray' within a cell, allowing real-time, continuous monitoring of complex signatures of infection. The goal of having an addressable array within a cell from which signals can be measured without perturbing cellular function may, for example, be enabled through the use of new nanomaterials that act as intermediaries between the biological system and the physical system used for measurement. Several types of nanomaterial, such as carbon nanotubes [22], gold nanoshells [23] and quantum dots [24], have unique electronic or optical properties that could be tuned to detect biomolecular concentrations within cells. In these examples, the nanomaterials may link with systems such as advanced silicon microelectronics or advanced imaging techniques such as fluorescent lifetime imaging microscopy (FLIM) or Raman microscopy.

There is a strong need for rapid, sensitive pathogen-detection systems that can be easily applied to the clinic, industry or even the 'battlefield'. It is important to acknowledge, however, that the transition of these technologies from the bench to real-world application depends on certain requirements. A number of groups have produced array data that could be used to produce viral barcodes or unique identifiers. These efforts, whether at the DNA or protein level, are currently disparate, uncoordinated and mainly confined to studies *in vitro*. A collection of such infection profiles, an Infection Profile Database, for example, needs to be put together that sets out standards and requirements that would help such high-dimensional data to be translated into clinical utility. Indeed, such information would provide a valuable resource for constructing specific cell-based sensors or even synthetic sensors.

One of the overwhelming problems related to the creation of unique signatures that will consistently and accurately identify an infection is the fact that the signatures depend on a large number of variables. A potential solution might involve identifying a signature that is produced early in infection and yet can be sustained for capturing later. This idea is perhaps not too far-fetched, and it may well involve certain immune cells, in particular those destined to become antigen-driven memory cells. Although various responses can be used to identify an infection, the heterogeneity of the system we propose (Figure 3) may be too variable, and thus the detection of these responses would require all patients to present within a very narrow characterized window for their output to be informative. In clinical terms, this scenario is obviously completely unrealistic. The question is whether infections leave early footprints that are unique and readable, or whether the response to infection as a whole is simply too dynamic. Answers to these questions are tractable but will require carefully controlled and appropriately powered studies as well as standardization of data measurements and quality assurance. Increasing attention is being given to these critical areas, and as a consequence we are in exciting times in this rapidly moving field.

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