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Microfluidics closes in on point-of-care assays

Peter K Sorger

An integrated microfluidic device enables rapid detection of protein biomarkers in patient samples.

Routine assessment of biomarkers promises to transform many areas of medicine, from clinical trials, which will benefit from biomarker-based segmentation of patient populations, to the diagnosis and treatment of complex disease[1]. To add real value, biomarkers must be measurable by clinicians and provide useful new information[2]. In this issue, Fan *et al.*[3] tackle the first of these challenges by developing a microfluidic integrated blood barcode chip that could serve as a point-of-care device for measuring multiple protein biomarkers in small samples of blood.

It is increasingly clear that the success of future therapeutics will depend on identifying those patients who are most likely to respond. Both payers and patients will demand evidence that drugs are working in specific individuals. Sophisticated biomarker analysis could contribute markedly to achieving these goals, improving the diagnosis, therapy and management of disease. Indeed, for good or ill, companies such as Biophysical in Austin, Texas, are already marketing panels of diagnostic biomarkers directly to patients.

Biomarkers include proteins, hormones or other biomolecules whose levels, states or activities change with disease state or treatment and provide diagnostic or prognostic information. Ideally, such biomarkers would be mechanistically linked to the etiology or pathobiology of the disease, but most biomarkers simply show tissue-specific patterns of expression or statistical correlation with a disease state. For example, prostate-specific antigen is a tissue-specific glycoprotein found on both transformed and normal prostate epithelia that has proven useful in monitoring cancer recurrence and, more controversially, in diagnosis[4]. However, moving biomarkers from the laboratory to the clinic has been remarkably difficult, in part because relatively large trials are required to assess their utility and because techniques that are routine in biomarker research, such as mass spectrometry, are not common in clinical laboratories (see ref. 5 for a discussion of the technical and commercial challenges). Such translational bottlenecks should be eased by the availability of simple and rugged biomarker assay devices[6].

Several features of microfluidic devices suggest that they would be ideal for point-of-care assays. These include their ability to process very small samples using small quantities of reagents (perhaps stored on-chip), to exploit the physics of low-Reynolds-number environments (such as free-flow electrophoresis) and small feature size (such as resonance-based mass sensing[7]) and to integrate multiple separators and sensors[8]. Integration is widely thought to be of particular importance, and the integrated blood barcode chip from Fan *et al.*[3] nicely demonstrates this capability (**Fig. 1**).

The chip first separates plasma from blood using microchannels and then assays for specific plasma components in the same channels. Isolation of plasma is achieved based on the tendency of cells to enter the higher-flow microchannel when a bifurcation is encountered (as first observed in rat capillaries[9]). On-chip separation of plasma from blood, previously described by Yang *et al.*[10], obviates the need for prior centrifugation of the sample. In the integrated blood barcode chip, the plasma-skimming channels contain successive rows of

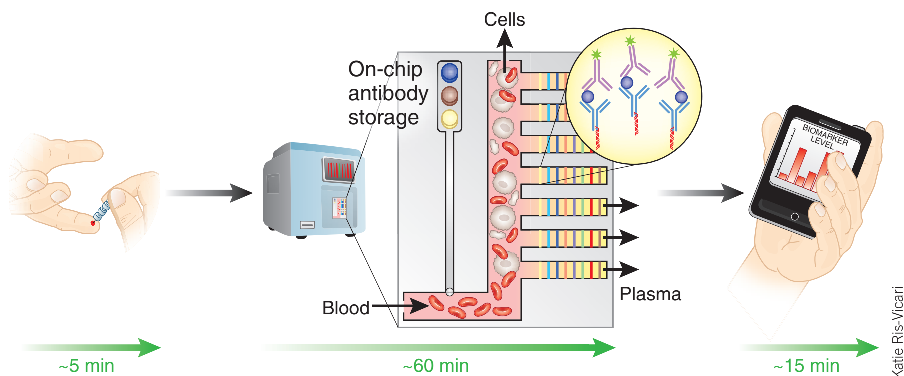

Figure 1 Possible workflow for a commercial microfluidic biomarker assay system. In devices such as the integrated blood barcode chip[3], chip regeneration and readout could involve an external workstation that includes pumps and wash buffers, although some chip-specific reagents might be stored on board. A blood sample collected with a standard lancet is introduced into the workstation and diluted with EDTA to prevent clotting. The blood is then introduced into the microfluidic chip, where it is separated into cells and plasma; in the integrated blood barcode chip cells go to waste, but they could be recovered for detection of genetic biomarkers and other purposes. Multiple analytes are then captured from plasma by patterned antibodies immobilized through DNA hybridization; alternatively, aptamers or other affinity capture reagents might be used. After washing and introduction of secondary reagents, analyte levels are read using the workstation. Biomarker measurements must be carefully integrated with patient history using sophisticated statistical algorithms because biomarker data are rarely simple or univariant. Ultimately, it would be desirable to produce devices in which more of the instrumentation was on board the chip so that a handheld device could be used to prepare and read the chip.

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immobilized antibodies that capture analytes present in the plasma for subsequent detection using fluorescent secondary antibodies.

Fan *et al.*[3] show that the levels of prostate-specific antigen detected in patient serum by their chip correlate well with those measured by conventional techniques, and the overall sensitivity of the device is sufficient to quantify multiple proteins and cytokines at levels relevant to human disease. However, whether fluorescence will ultimately be the best way to detect biomarkers remains unclear; a wide variety of detection methods are under development, including several that are label-free[7].

Nearly a decade after widespread clinical use of microfluidics was first anticipated, the field remains plagued by technical problems. One issue concerns the sensitivity and durability of antibody arrays, on which most biochemical assays rely. The integrated blood barcode chip addresses this problem by using DNA-directed protein immobilization[11], in which a surface coated with single-stranded DNA is hybridized to antibodies chemically conjugated to complementary DNA. This seemingly baroque scheme has important advantages over direct antibody conjugation, including reliance on robust and stable DNA-glass surfaces during device fabrication and storage, formation of delicate antibody-containing features just before use, and creation of capture features with densely packed antigen-combining domains, thereby increasing sensitivity[12].

Passivation and the prevention of fouling are two additional challenges in using microfluidic devices with biological samples. Passivation refers to the modification of surfaces so as to minimize nonspecific binding, and fouling to unwanted binding to device surfaces and to plugging of microchannels by components of the sample. Fouling leads to irreproducible measurements and device failure. Because most microfluidic devices based on soft lithography (such as the integrated blood barcode chip) are bonded to glass, and glass has a very high nonspecific binding capacity for proteins, lipids and other biomolecules, effective passivation is difficult to achieve. Perfect device passivation and prefiltering of samples, which would solve the problem of fouling, has never been achieved. Fortunately, the multilayer polyamine-DNA-antibody surfaces created by DNA-encoded antibody immobilization are anti-fouling and appear to be very effectively passivated. The remaining fouling problems relate to background that limits the ultimate sensitivity of the device.

Fan *et al.*[3] demonstrate impressive sensitivity with their chip, but largely using serum (plasma cleared of fibrinogen and other clotting factors) rather than plasma itself, which gives much higher nonspecific binding than that of serum[13]. Further experiments with fresh blood from healthy volunteers and patients, as outlined by the authors, should resolve whether the rapid processing of blood made possible by their chip will solve the sensitivity problems that have impaired less sophisticated devices.

It is now clear that the micro total analysis system (μTAS or lab-on-a-chip) proposed over a decade ago[14] will be substantially harder to achieve than was first envisioned. Indeed, several companies founded on the μTAS concept have already faltered or failed. Looking forward, we can nonetheless expect steady improvement in microfluidic technologies, antibody- and aptamer-based capture of biomolecules, label-free detection and on-chip integration of multiple preparative and analytical units with diverse functions. It seems highly likely that devices such as the integrated blood barcode chip presage commercial analytic devices whose reliability, simplicity and low cost will revolutionize the use of protein biomarkers.

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MicroRNAs fine-tune oncolytic viruses

John C Bell & David Kirn

Targeting by tissue-specific microRNAs enhances the efficacy and safety of tumor-killing viruses.

Gene silencing by endogenous microRNAs (miRNAs) has recently been exploited to control the tropism of gene-therapy vectors. By including the target sequence of a tissue-specific miRNA in the genome of lentiviral vectors, Naldini and colleagues suppressed transgene expression specifically in selected cell types such as hematopoietic cells and hepatocytes[1,2]. This strategy has now been extended to control targeting of oncolytic viruses. The new studies, appearing in *Molecular Therapy*[3], *Journal of Virology*[4] and *Nature Medicine*[5], mark the beginning of innovative efforts aimed at discovering combinations of viruses and miRNA targets that yield safer and more effective anticancer virotherapeutics.

miRNAs are versatile, noncoding RNAs, ~22 nucleotides in length, that exert post-transcriptional regulation through specific recognition of short sequences, often located in the 3′ untranslated region (UTR), in target mRNAs[6]. Depending on its degree of complementarity to the target, the miRNA can affect either the stability or translation of the mRNA[7]. miRNAs have complex expression profiles that reflect the important roles they play in the control of mammalian growth and development. The human genome is estimated to contain >500 miRNA genes. Some are expressed in a tissue-specific fashion, whereas others are constitutively expressed or turned on in response to endogenous cues or stress signals[8]. Another important function of miRNAs, clearly demonstrated in plants and invertebrates[9], is to suppress viruses by binding to cognate sequences in viral mRNAs.

Oncolytic viruses are vectors engineered or selected to infect and kill cancer cells while leaving normal cells relatively unscathed[10,11]. Clinical data show that this class of therapeutic is safe and cancer selective but also suggest that more potent viruses—targeted specifically to malignancies—would be desirable to

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