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Point-of-care biosensor systems for cancer diagnostics/prognostics

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Abstract

With the growing number of fatalities resulting from the 100 or so cancer-related diseases, new enabling tools are required to provide extensive molecular profiles of patients to guide the clinician in making viable diagnosis and prognosis. Unfortunately with cancer-related diseases, there is not one molecular marker that can provide sufficient information to assist the clinician in making effective prognoses or even diagnoses. Indeed, large panels of markers must typically be evaluated that cut across several different classes (mutations in certain gene fragments—DNA; over/under-expression of gene activity as monitored by messenger RNAs; the amount of proteins present in serum or circulating tumor cells). The classical biosensor format (dipstick approach for monitoring the presence of a single element) is viewed as a valuable tool in many bioassays, but possesses numerous limitations in cancer due primarily to the single element nature of these sensing platforms. As such, if biosensors are to become valuable tools in the arsenal of the clinician to manage cancer patients, new formats are required. This review seeks to provide an overview of the current thinking on molecular profiling for diagnosis and prognosis of cancers and also, provide insight into the current state-of-the-art in the biosensor field and new strategies that must be considered to bring this important technology into the cancer field.
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1. Introduction

Cancers arise as a result of the disruption of normal cell signaling pathways, which can produce cells (cancer cells) that exhibit a decisive growth advantage compared to their neigh-

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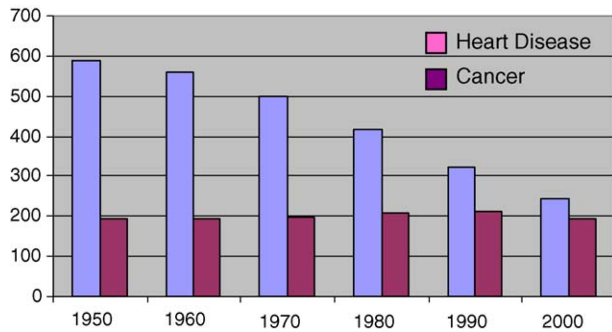

Fig. 1. Age-adjusted death rates from heart disease and all cancer-related diseases from 1950 to 2000.

bors. These growth advantages are typically produced from a number of different genetic and/or epigenetic changes, which result in the activation of oncogenes and the inactivation of tumor suppressor genes. Unfortunately in terms of diagnosis, no single oncogene or tumor suppressor gene has been discovered to be universally altered in all adult cancers. In addition, patterns of genetic and/or epigenetic changes differ not only in terms of tumor location (i.e., organ), but also among tumors from the same location. Besides genome-related changes, other complex molecular alterations result during the course of tumorigenesis, such as gene over/under-expression (mRNA changes) or protein over/under-expression. As such, a plethora of molecular biomarkers can potentially be analyzed via different sensing platforms for tumor classification to guide diagnosis, prognosis, monitoring treatment and disease recurrence.

As depicted in Fig. 1, the age-adjusted death rate for heart disease has dramatically declined over the last 50 years, while in the case of cancer this same trend has not occurred (Leaf, 2004). In 2004, nearly 563,700 patients were diagnosed with one or several of the 100 diseases belonging to the cancer family with one in two and one in three men and women, respectively, expected to contract one of these diseases during their lifetime. It is interesting to note that ∼90% of all cancer-related deaths occur from metastasis and not directly from the primary tumor site.

In spite of the rapid explosion of new technology platforms and biomarkers that have been discovered and reported in the literature for cancer diagnostics, prognostics, therapeutics and monitoring disease recurrence, few of these technologies or biomarkers have transitioned into the clinical arena. The common method for cancer diagnosis and prognosis relies heavily on technologies that are over 100 years old (paraffin fixation of tissues with visual inspection of cell morphology by a pathologist). Therefore, in spite of the significant investment by a number of agencies into discovery of new molecular markers and the technologies to utilize these biomarkers, most have not entered the clinic. The major fundamental question then arises: Why have the incidence and survival rates of cancers not shown marked decreases in line with the large financial and time investments that have been waged against this disease? In this paper, information will be presented that provides information on potentially new technologies in the form of point-of-care (POC) biosensors for biomarker analysis and the merging of new biomarkers with the appropriate technology platform to develop systems that provide clinically relevant information to assist the physician and clinician in disease diagnosis, prognosis, treatment and recurrence. The major technology platform that will be the focus of this discussion is biosensors, and their integration into POC systems for the analysis of clinically significant cancer biomarkers.

2. Biosensors and point-of-care technologies

2.1. Description of technology area

A biosensor (see Fig. 2) in the traditional sense is defined as: bioanalytical device incorporating a biological material or a biomimic (e.g., tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, etc.), intimately associated with or integrated within a physicochemical transducer or transducing microsystem, which may be optical, electrochemical, thermometric, piezoelectric or magnetic. The usual aim of a biosensor is to produce either discrete or continuous digital electronic signals, which are proportional to a single analyte or a related group of analytes.

POC systems are viewed as integrated systems that can process clinical samples for a number of different types of biomarkers in a variety of settings, such as clinical laboratories, doctors' offices and eventually, at home. *Basically*, *POC systems make state-of-the-art technology platforms accessible to a large population pool*. From a diagnostic or prognostic perspective, POC systems provide the clinician the ability to have access to a wealth of molecular information for providing profiles of cancers using novel technology platforms that in the past have been accessible to only major cancer centers. The development of POC technologies will provide opportunities for better screening of at-risk patients, tighter surveillance of disease recurrence and better monitoring of treatment. In addition, POC technologies are by their very nature, low cost in their implementation making large scale screening for disease prevention more attractive to health care insurers.

2.2. Biomarkers for cancer

The utility of any biosensing platform is intimately dependent on the viability of biomarker(s) for producing diagnoses with high confidence. In particular, biomarkers must not only signal the presence of a tumor or cancer, but should also predict the

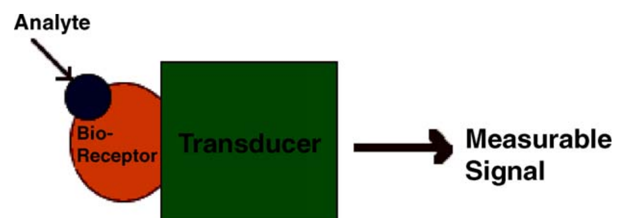

Fig. 2. Schematic representation of a single element biosensor containing the biorecognition element, transducer and the physical output whose magnitude is related to the concentration of the analyte of interest.

stage of tumorigenesis. The explosion in molecular biology and associated technologies has led to a much better understanding of human cancer and malignancy and potential biomarkers that can be used for diagnosis. In spite of these advances, progress in identifying and applying useful diagnostics in the cancer arena has been slow. For example, the number of markers recommended for routine clinical use in colorectal and breast cancer by the Tumor Marker Guidelines Committee of the American Society of Clinical Oncology is remarkably short. An understanding of why these guidelines are so conservative sheds insight into hurdles and challenges for development of cancer diagnostic platforms (Hayes et al., 1996).

1. *What is the potential utility*? A new biomarker might have one or more utilities, each requiring a separate set of investigations. These utilities include risk categorization, screening, differential diagnosis, prognosis of early or metastatic disease, prediction of benefit from specific therapies, and monitoring for early relapse or of clinical course in patients with established metastatic disease.
2. *What are the technical performance characteristics of the assay*? One of the biggest problems in marker implementation is reproducibility of the assay, or between assays, for a given cancer-related change. Frequently, from one study to the next, there are many different assay formats for a given marker, or within the same format there might be different reagents, or even using the same reagents there might be different analytical criteria and cut-offs for "positive" versus "negative".
3. *Does the marker distinguish two populations so clearly that different clinical decisions would be made*? For example, several studies have documented that up to 50% of patients with newly diagnosed breast cancer would take adjuvant chemotherapy if the chances of improving their survival over 10 years was as little as 1%. In this case, a marker would have to be exquisitely and absolutely accurate in identifying those unlikely to benefit (either because of prognosis or prediction) from those who would. This consideration differs from situation to situation, depending on disease status and drug toxicity.
4. *Clinical context*: Does the detection of the analyte result in sufficiently "robust" differences in outcomes? Is that difference reliably estimated (statistics emphasize that a p-value of <0.05 does not make the marker clinically useful, it only implies that the separation between the two groups is likely to be real, even if it is small). Does knowledge of that difference make a clinical difference? Are therapies available that would be applied in the presence or absence of marker positivity and that would NOT be in the reverse circumstance?
5. *Why is a marker or test accepted or not*? Three reasons, all based on the considerations above, but not necessarily overlapping: (1) guidelines/expert opinion (panels or individuals). These groups are usually, but not always, evidence based, and they depend on data that indicate that knowledge of the assay results would lead to a change in therapy that has been demonstrated to improve clinical outcomes (Bast et al., 2001). (2) Regulatory bodies (FDA, CLIA). These bodies have usually NOT considered outcomes as endpoints to determine clearance. Rather, they have based their deliberations on whether the assay is effective in detecting the analyte accurately and reproducibly. Furthermore, FDA approval is not required for marketing of a marker, if the vendor uses it as a "home brew," although in this case CLIA approval is required. (3) Reimbursement (Government (CMS), third party payers, individual patients). FDA clearance has been considered but has not necessarily been a criterion for reimbursement.

As an example of the challenges associated with biomarkers in cancer management and diagnosis, few markers have completely filled the above mentioned criteria for any utility of the known malignancies. A number of genetic abnormalities, such as germline RB, p53, BRCA I & II, APC and MMR genes, have been found to be accurate predictors of *risk* of certain malignancies, but they are uncommon in the general population and therefore not very helpful. Weaker and less penetrant susceptibility genes have, thus far, not been helpful as well. The only *screening* techniques that are proven or felt to lower mortality are PAP smears (cervical), colonic fecal occult blood/sigmoidoscopy/colonoscopy (colorectal), mammography (breast), and perhaps prostate specific antigen (prostate).

Reliable biomarkers of *prognosis* that might influence treatment decisions include chromosomal abnormalities in selected leukemias, and arguably, circulating alpha-fetoprotein and beta-human chorionic gonadatropin in males with germ cell (testicular) tumors. Clinically useful *predictive* markers are more common, and include certain chromosomal translocations for prediction of all-trans retinoic acid or imatinib in leukemias and expression of targeted growth factor receptors in selected solid tumors, especially regarding HER2 and trastuzumab in breast cancer. Finally, markers that permit *monitoring* of patients with advanced disease to provide an indication of treatment success or failure in the presence of alternative therapies that might be offered have found utility in many solid tumors, especially colon (CEA), ovarian (CA125), prostate (PSA) and breast (CA15-3 or CA27.29 and perhaps enumeration of circulating tumor cells).

There are several reference sites available from which to gather relevant literature on existing and evolving biomarkers for the various cancer diseases. For example, the National Center for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov), which was established in 1988 as a national resource for molecular biology information, contains genome-related information associated with different diseases.

3. Novel technologies for POC biosensing platforms for cancer diagnostics

POC technologies offer platforms for complex testing by non-specialists and the benefit of increased robustness and reliability due to fewer world-to-device interfaces. These technologies can contribute to the realization of personalized medicine by creating a link between the diagnosis of disease and the ability to tailor therapeutics to the individual. As biomarkers of disease are discovered and validated through genomics and proteomics

research, development of new technology platforms can enable rapid introduction of these discoveries into clinical practice as well as aiding in biomarker discovery efforts.

3.1. State-of-the-science

The basic building blocks of any biosensing platform consist of the biorecognition element, the transducing element and the readout modality. However, as noted above, the biosensing platform for cancer diagnosis/prognosis may be a bit more involved due to the complexity of these diseases. Therefore, integration of the biosensor into a functional POC system may be required to provide initial front-end sample preparation. As such, the basic components of the biosensor must be amenable to integration into the POC system and provide multiplexing capabilities if the technology is to be appropriate for cancer diagnostics and prognostics. In the sections that follow, key elements that comprise a functional biosensor will be discussed as well as recent innovations in these component areas and their integration into the cancer diagnostics and/or prognostics arena.

3.2. Molecular recognition elements

The ability to recognize the "target" or biomarker in a mixed population is viewed as the critical step in any diagnostic assay. The biomarker can be present intracellularly or extracellularly. Whatever the sampling matrix, the biomarker must be recognized and collected from a heterogeneous population. In addition, the marker can be as complex as a whole cell or as simple as a single molecule (antigen like the prostate specific antigen). While antibodies can be used for recognizing these biomarkers, more recently, synthetic based recognition elements are being investigated as replacements for antibodies, such as aptamers, peptides, surface-imprinted polymers or molecularly imprinted polymers. Listed below are some examples of recognition materials.

(a) The most common approaches for generation of cell-specific or molecular ligands are to select a single, well-characterized biomarker and generate antibodies or screen libraries for components that bind to it. In the case of generation of cell-based ligands, prior knowledge of the individual cellular components is necessary, which may require production or purification of the target protein. Most efforts in this field have focused on exploiting monoclonal antibodies as specific ligands (Farah et al., 1998; Glennie and Johnson, 2000; Green et al., 2000; Pietersz and McKenzie, 1992). Polyclonal antibodies are typically generated against a single antigen and a large body of work is usually required to select the "best" antigen. However, recent advances have been made in high-throughput polyclonal antibody generation (Chambers and Johnston, 2003). If a continuous source of the specific antibody is needed, a monoclonal antibody may be produced.

(b) For cell-based recognition, little is typically known about the cellular landscape of the target cell making rational design of cell-targeting ligands impossible. Thus, high-throughput methods are required to screen libraries of potential ligands for cell binding, even when the nature of the cell surface is undetermined. Using phage display libraries, protocols to identify cell-specific binding peptides has been developed (Barry et al., 1996; Brown, 2000; McGuire et al., 2004a,b; Oyama et al., 2003). A remarkable feature of the peptides selected using this procedure is their cell-specificity. This high discriminating power of the selected phage suggests that peptides could be identified that selectively bind to different tumor types, even those with similar classifications. The approach employs an unbiased screen in which there is no selective pressure towards binding a particular macromolecule. This has the important advantage that it requires no prior knowledge of the cellular receptor.

(c) Several synthetic ligands have been studied for a variety of targets, such as aptamers and peptides. The particular advantages of these synthetic ligands as opposed to antibodies is that they are robust structures that can be placed in diverse settings without losing their specificity, can be made by conventional "wet" chemistry techniques and are easily modified structurally to support the addition of reporters or immobilization to sensing elements.

  (i) Peptides are smaller than antibodies, can be chemically synthesized in large quantities and are amenable to derivatization. Furthermore, peptides can display high affinities for cell surface components, making them attractive ligands for cell recognition. Phage display has been used to identify peptide ligands that target certain cell types by panning on well-characterized cell surface receptors. In most cases, a purified tumor-associated protein has been used as the bait, and peptides are isolated by panning on the purified protein (Burg et al., 1999; Gui et al., 1996a,b). For exampurified $\alpha_V\beta_3$ integrin, a protein marker for angiogenic endothelium (Pasqualini et al., 1997). More recently, peptides with affinity for the tumor antigens HER2/neu receptor, oligonucleotide receptor, ErbB-2, and ICAM-1 have been isolated by phage display (Belizaire et al., 2003; Karasseva et al., 2002; Schatzlein et al., 2001). Peptide-bead libraries can also be screened for ligand binding (Aina et al., 2002; Mikawa et al., 2004) and peptides have been isolated that recognize the $\alpha_6\beta_1$ integrin expressed on the prostate cancer line DU145 (DeRoock et al., 2001).

  (ii) Aptamers can bind to their targets with high affinity and even discriminate between closely related targets. This is due to their adaptive recognition: aptamers, unstructured in solution, fold upon associating with their targets into molecular architectures in which the ligand becomes an intrinsic part of the associated structure. The main advantage over antibodies is overcoming the use of animal systems for the production of the molecule. In addition, aptamers can be designed in a signaling architecture, in which the structure of the aptamer changes upon association producing a signaling event due to the molecular association. Aptamers are isolated by in vitro methods that are independent

of animals and can be generated against any target; the immune response to generate antibodies can fail when the target molecule (a protein) has a structure similar to endogenous proteins and when the antigen consists of toxic compounds that can even kill the animal. The production of aptamers is commonly performed by the systematic evolution of ligands by exponential enrichment (SELEX) process (Brody et al., 1999; Cerchia et al., 2002; Eaton et al., 1997; Ulrich et al., 2001).

3.3. Optical transduction of association events

Various optical transducers have been exploited in biosensors including fluorescence spectroscopy, interferometry and spectroscopy of guided modes of optical waveguides and surface plasmons. Fluorescence has been a mainstay in the biosensing arena due to:

(a) the wide availability of labels that cover a large spectral range and different functional groups;
(b) reduction in size and operational complexity of laser-induced fluorescence hardware;
(c) the ability to achieve single molecule detection sensitivity.

While fluorescence is and continues to be a major transduction modality in many biosensing platforms and systems using optical readout, alternative technologies are evolving as well. An example is sensors based on spectroscopy of guided modes of optical waveguides (grating coupler, resonant mirror) and surface plasmons, which can be considered as label-free measurement technologies. Sensors based on spectroscopy of surface plasmons, sometimes referred to as surface plasmon resonance (SPR) sensors, represent the most advanced type of label-free sensors based on spectroscopy of guided waves. Numerous configurations of SPR sensors have been proposed and developed (Jordan and Corn, 1997; Lee et al., 2001; Nelson et al., 2001, 2002; Smith and Corn, 2003; Thiel et al., 1997). The first commercial SPR biosensor was launched by Biacore International AB (Biacore) and in the following years, Biacore and other companies (Nippon Laser and Electronics Laboratory, Texas Instruments, etc.) have produced several SPR instruments. SPR sensors for detection of numerous important substances have been demonstrated, including hormones (luteinizing hormone, human chorionic gonadotropin), toxins (fumonisin B1, for staphylococcal enterotoxin B, botulinum toxin and *Escherichia coli* enterotoxin), and bacterial pathogens (*E. coli*, *Salmonella enteritidis* and *Listeria monocytogenes*).

3.4. Piezoelectric transduction of association events

Another technology for transduction in biosensing platforms is piezoelectric, which also possesses the advantage of not requiring the use of labels and can record in real-time the affinity reaction allowing kinetic studies (Liu et al., 2003). Piezoelectric affinity biosensors are based on the coupling of the biological recognition element with a piezoelectric element, usually a quartz crystal coated with gold electrodes (Babacan et al., 2000; Tombelli et al., 2002). Piezoelectric crystals have been used as microbalances and as a microviscometer owing to their small size, high sensitivity, simplicity of construction and operation, light weight and the low power required. The quartz crystal microbalance (QCM) has traditionally been used in many applications such as thin film deposition control, etching studies, aerosol mass measurements and space system contamination studies (O'Sullivan and Guilbault, 1999). The first report on the direct detection of nucleic acid interactions based on the use of acoustic wave devices was provided by Fawcett et al. (1988). Since this early work, a number of articles have appeared employing similar procedures, resulting in microgravimetric measurements of nucleic acids (Caruso et al., 1997; Fawcett et al., 1998; Mannelli et al., 2003; Minunni et al., 2003; Willner et al., 2002; Zhou et al., 2001, 2002). A large amount of work in this field has been produced by Thompson and co-workers who investigated RNA–protein (Furtado et al., 1999), DNA–DNA (Furtado and Thompson, 1998) and RNA–RNA (Su et al., 1997) interactions.

3.5. Quantum dots for readout of association events

The emergence of luminescent nanocrystals (quantum dots) as a viable alternative to molecular labeling fluorophores in biological applications has opened the way to new cellular biological studies (Chen and Rosenzweig, 2002; Gerion et al., 2002, 2001; Kim et al., 2004; Kricka, 2002; Maxwell et al., 2002). In these studies, luminescent quantum dots can be attached to protein molecules for intracellular tracking or for transducing molecular association events. Another example is the use of luminescent quantum dots to label antibodies against membranal antigens. In the case of breast cancer, the preparation of quantum dot bioconjugates of anti-HER2/Neu, anti-PgR, anti-Mammaglobin, anti-cycline E and anti-uPA could prove useful in the detection of rare cancer cells. Quantum dots show several distinct advantages over organic fluorophores due to their unique spectroscopic properties. Their narrow emission peaks will enable spectral multiplexed analysis. Their broad excitation spectrum will permit as well the excitation of multiple emission-colored quantum dots with a single excitation wavelength. Their exquisite photostability will enable longer observation times of, for example, labeled cells without noticeable bleaching. Finally, their high emission quantum yield will improve the signal to noise ratio in most measurements, which will in turn decrease false positive and negative readings. There are several challenges and problems that need to be addressed to successfully utilize quantum dots in cancer diagnostics:

(a) improved methods for the synthesis of quantum dots with high stability in aqueous media and more importantly biological fluids (serum, plasma, growth medium);
(b) improved capping of quantum dots to prevent aggregation;
(c) development and optimization of the conditions to realize stable and active quantum dot bioconjugates;
(d) theoretical understanding of the parameters affecting FRET efficiency between quantum dots and fluorescent acceptors or quenchers;

(e) development of the protocols necessary to fabricate biochips with deposited luminescent quantum dots.

3.6. Electrochemical transduction of association events

Electrochemical biosensors are extremely useful for delivering biodiagnostic information in a fast, simple, and low cost fashion in connection to POC analyzers. User-friendly self-testing glucose strips, based on screen-printed amperometric enzyme electrodes, have revolutionized the field of diabetes management and have come to dominate the US$ 5 billion/year diabetes monitoring market since their launch 18 year ago. The i-STAT hand-held battery-operated clinical analyzer, combining different amperometric biosensors and potentiometric ISE (on a single disposable cartridge), has been widely used for rapid POC measurements of multiple electrolytes and metabolites in emergency settings (http://www.i-STAT.com). Nanoscale materials offer excellent prospects for designing powerful bioanalytical protocols with remarkable sensitivity and multiplexing/coding capability (Gau et al., 2003; Jain, 2004; Sun et al., 2005). The enormous signal enhancement associated with the use of nanoparticle amplifying labels and with the formation of nanoparticle-biomolecule assemblies provides the basis for ultrasensitive electrochemical detection. Bioaffinity assays on nanowire transducers offer great promise for label-free simplified detection of biomolecular interactions (Huang and Lieber, 2004; Wang et al., 2005; Woolley et al., 2000).

3.7. Integrated systems for complex biosample processing

Micro-total analysis systems (μTAS): an extension of the basic sensor by providing a workable platform for the biosensor is the micro total analysis system (or "lab-on a-chip"), which is a device that integrates multiple bioanalytical functions into a small, portable instrument, essentially incorporating functions of a full-sized laboratory into a miniaturized device with microscale or nanoscale components (Ahn et al., 2004; Burns et al., 1998; Gambari et al., 2003; Roper et al., 2005; Rudert, 2000; Wang, 2000; Wang et al., 2003). The potential benefits of μTAS include limited reagent use, faster sample analysis, higher throughput, increased sensitivity, portability, automation of complex bioanalytical processes, ease of use, and closed architectures for minimizing false positives arising from sample environmental contamination. Some examples of μTAS are listed below.

(a) The Lab-on-a-Disc concept has been pursued for over a decade, first through fluorescence detection from micro-spots (Ekins and Chu, 1991), then adding centrifugal microfluidics for reagent distribution (Gyros, http://www.gyros.com), and most recently for surface-normal interferometry (La Clair and Burkart, 2003). The centrifugal action is not fundamentally different from pressure-based microfluidics, but does provide ease of operation without the need for attached pressure or vacuum lines. The more important aspect of the disc is the rapid sequential reading of assay spots due to the rapid rotation of the disc. The read-out speed is most evident in the case of the BioCD (Varma et al., 2004a,b). The discs spin at 6000 rpm delivering up to 5 billion individual assay spots to the interrogating laser in under 10 min. The speed of the BioCD is based on the high photon flux of interferometric (direct) optical detection, which can be three to six orders of magnitude larger than for fluorescence detection.

(b) The common substrate material used for developing μTAS has been glass and/or quartz due to their well-established microfabrication techniques to make the prerequisite structures (optical lithography followed by wet-chemical etching), their favorable optical properties and a diverse range of surface modification protocols. However, fabricating μTAS for clinical applications, which demand disposable formats for eliminating false positives due to sample carryover, is problematic using these materials due to the serial nature of chip fabrication and the high cost of the devices. As an alternative, polymer-based substrates are viewed as a viable material since microchips can be fabricated using a variety of different micro- and nanofabrication technologies that are conducive to mass producing integrated systems at low costs (Barker et al., 2000; Becker and Locascio, 2002; Buch et al., 2004; Ford et al., 1999; Lai et al., 2004; Noerholm et al., 2004; Rossier et al., 2002; Soper et al., 2000). For example, exquisite micro- and nanostructures can be produced from appropriately prepared masters using such techniques as injection molding, nanoimprint lithography (Chou et al., 1996; Falconnet et al., 2004; Heidari et al., 1999; Tan et al., 1998) or hot-embossing in high volume and at low cost. In addition, a variety of simple and robust modification chemistries are being developed for polymers that allow, for example, the immobilization of biorecognition elements to the surface of fluidic networks (Fixe et al., 2004; Henry and McCarley, 2001; Henry et al., 2000; Johnson et al., 2001).

(c) System integration to create a 'bleed and read' diagnostic microdevice must contain multifaceted functionality to address sample acquisition, sample preparation, sample analysis and data processing. The need for sample preparation emanates from the fact that diagnostic analysis requires more than an analytical measurement step—preparation to transform the sample from its native form into one that is amenable to analysis. For genetic analysis, both DNA purification and amplification are key processes needed for extracting genetic information from the sample. Reasonable progress has been made with DNA purification via solid phase extraction (SPE) on silicon posts (Christel et al., 1999), silica beads, silica sol–gels (Wolfe et al., 2002) and combinations of the latter two (Breadmore et al., 2003). While these approaches are capable of reasonable extraction efficiencies (50–70%), issues exist with capacity and no chip-based method has matched the performance of the commercial macroscale systems (e.g., Qiagen spin tubes). For DNA amplification, a variety of methods have begun to show promise. Rapid glass-microchip-based temperature cycling has been accomplished by a number of heating methods that include resistive heating (Lagally et al., 2004), inductive (Pal and Venkataraman, 2002), infrared (Giordano et al., 2001)

and continuous flow (Hashimoto et al., 2004). Integration of multiple functionalities into a single device will require valving to control flow through the microfluidic architectures and isolate the individual units or functional domains of the chip. Two premier valving methods have surfaced from Quake (Unger et al., 2000) and Mathies (Skelley et al., 2005).

4. Summary and conclusions

Extensive work within the physical sciences and engineering fields has resulted in the evolution of a variety of new tools that are well-equipped to monitor the presence or absence of certain biomarkers across a number of different classes (DNA, RNA and proteins). Indeed, many of these devices have already been employed in the clinic for the diagnosis and/or prognosis of certain diseases. However, the use of these sensing platforms for cancer has been slow to evolve due primarily to the complexity of these diseases, requiring the need for monitoring simultaneously a large panel of biomarkers and the necessity for multi-step sample processing. Therefore, the integration of classical biosensing platforms into integrated systems, such as lab-on-a-chip devices, may be required for these technology platforms to enter the clinical cancer field. A particularly attractive focus is to develop POC devices for providing greater accessibility of these technologies for large-scale screening to assist in diagnostics. Also, the ability to transition new innovations in biosensors and POC devices into the clinic will depend heavily on the acceptance of existing molecular biomarkers into the clinic as well as the discovery of new biomarkers.

5. Future perspectives and technology needs for meeting the demands of cancer diagnosis/prognosis

While much research has evolved as a result of opportunities from a variety of agencies, including the NIH, it is clear that new technology-focused funding opportunities will have to be developed to produce a new wave of biosensors that are appropriate for providing clinically relevant information for the 100 or so cancer-related diseases. During the evolution of biosensor related platforms for providing clinical information for cancer patients, the following issues should be kept under consideration:

(a) *Utility of biosensors*: Biosensors must be used to detect and quantify the presence of biomarkers for cancer to aid clinicians in determining the type of cancer based on a molecular signature or a panel of signatures, and also in determining the stage of the disease and in selecting the most appropriate therapy. Targeted therapies are likely to become more prevalent and they will require patient specific biomarker detection and quantification to ensure selection of the correct therapy.

(b) *Patterns*: Molecular signatures of cancer are likely to be represented by expression level patterns of many different biomarkers. A wealth of markers is a likely outcome of all the functional genomics, proteomics and systems biology research that is currently being conducted. Therefore, the biosensor must not only signal the presence/absence of a particular biomarker, but provide quantitative information over a large dynamic range for many different biomarkers.

(c) *Sample media*: These biomarkers can be found in tissue, serum, plasma and urine and possibly in saliva as well. All of these sample matrices are complex media with many different types of biomolecules with a wide range of expression levels. Therefore, a high level of specificity is required of the biosensing platform to analyze the target(s) with minimal interferences from endogenous components present in the sample matrix.

(d) *Tissue samples*:
   (i) When the sample is from a biopsy, it is critical to the acceptance of new technologies that the biosensor add to the diagnostic process and not require the replacement of any current practices.
   (ii) In almost all cases it is very important that the biosensor technology be capable of analyzing very small samples as the volume of tissue available is often very small. For example, fine needle biopsies, which can provide ∼100 cells or so for interrogation. The ability to analyze one, or a few cells captured by either flow cytometry or laser capture microdissection would be exciting.

(e) *Detection limits*: It is likely that the concentration of biomarkers in either serum or urine will be very low. This will require that biosensors for cancer diagnostics will have to possess excellent sensitivity. It also imposes very stringent specificity requirements for the ligands that are used as the biological recognition elements. In the case of intracellularly confined biomarkers, high sensitivity will be required as well, since dealing with small clinical samples inevitably means low or even single target cells that must be analyzed.

(f) *Cost*: For biosensor technologies to be placed in POC settings, both the fixed and operational costs must be low. The instruments used with the biosensors must be inexpensive enough that the reagent rental model can be applied. The consumable costs must also be low if a technology is going to be used on large numbers of patients for screening.

(g) *Screening*: Great care must be taken with screening technologies. They must be foolproof, very inexpensive and deliver high fidelity results. A good place to introduce screening technologies is in patients that have been treated for cancer and are in remission to potentially monitor disease recurrence.

(h) *False positives versus false negatives*: Both are bad. In the case of false negatives a patient may be sent away with a clean bill of health when treatment is called for. In the case of a false positive, the patient experiences a stressful period that is hopefully corrected with more extensive testing.

(i) *Real-time detection perioperatively*: The ability to provide surgeons with a diagnostic tool that can identify the margins of a tumor is currently an unmet need.

(j) *Integration with other devices*: The integration of biosensors into other sample handling and processing devices will expand the utility of both technologies.

(k) *Creation of companion devices for use in research*: Much early discovery work involves the use of small animals. With these animals the amount of sample, tissue or body fluids that can be extracted is limited. If analysis tools can be developed that can handle extremely small sample volumes, the pace of discovery can be accelerated and the research diagnostic can be co-developed with the therapeutic.

Several enabling tools are viewed as important developments into biosensing platforms for cancer diagnostics and prognostics, and a few are listed below.

(a) *Integrated systems for POC applications*: Recent advances in the design and microfabrication of electronic, optical, mechanical and fluidic components for microelectromechanical and microfluidic systems have enabled fundamental studies of biosensing platforms. But a challenge remains regarding incorporation of the respective components into fully integrated systems that can handle all aspects of analysis of complex, clinically relevant biological samples without undue reliance on external macroscale systems.
(b) *Systems for single-cell analysis*: With the focus of current research shifting to the analysis of increasingly complex samples, applications in the area of single cell analysis are beginning to emerge that have the potential to shed light on the heterogeneity of biological and disease processes found in populations of cells. The microfluidics scale is well-suited to cell handling and analysis, with lab-on-a-chip technologies providing capabilities in cell trapping, cell sorting, cell culturing and analysis of intracellular components. But with single cell analysis, there is the added challenge of working with very small volumes and limited numbers of biomolecules within a given cell. With advances in this area, especially with respect to high throughput capabilities and the appropriate utilization of nanotechnologies, single cell analysis could become a useful tool for diagnosing disease at an early stage at which changes on a tissue level are not yet evident but chemical changes within cells are observable.
(c) *Systems with high multiplexing capabilities*: It is clear that there is not one biomarker that can provide detailed clinical information on all of the ~100 cancer-related diseases. In addition, the clinical information that is required is not simply the fact that the patient does or does not have the disease (diagnosis), but also other pertinent information as well, such as the stage of tumorigenesis, appropriate course of treatment (personalized medicine), margins if surgical resection is required, monitoring course of treatment and finally, monitoring for disease recurrence. Detailed molecular profiling to provide such exhaustive information cannot arise from just one class of markers, but most likely from a large panel of markers that cut across several different classes, such as genomic DNA (mutations—scanning and detection), mRNA (gene products—over- or under-expression) or proteins (over- or under-expression). Therefore, new technology platforms must possess the capability to provide data on a large panel of markers (multiplexing capability) across several different classes of biomarkers.

6. FDA and the regulation of in vitro diagnostic devices (IVDs)

The efficient translation of new technologies into clinical use is benefited by the early involvement of the FDA. FDA began regulating medical devices, including IVDs or laboratory tests, with the passage of the Medical Device Amendments of 1976. This new law put into place a series of general controls for medical devices including the requirement that companies register and list their products with FDA, make these products consistently over time following good manufacturing practices, and report post market adverse events to FDA. As a result of this legislation, for the first time in history FDA had a menu of laboratory products being sold in the US medical marketplace, tools for assuring companies made these consistently over time, and a system for identifying device problems in real world use and working with companies to remedy possible non-compliance. The 1976 Law also put into place new requirements for pre-market review of medical devices as well, which was based on device risk. The major risk posed by IVDs is related to the informational content they provide and the clinical response to true versus false positive and negative results.

Over the past decade since passage of the Modernization Act of 1997, FDA has worked hard to develop regulatory tools to foster rapid transfer of new technology from the research bench into clinical practice. The Modernization Act emphasized the need for FDA to maintain a "least burdensome" approach toward its review practices (maintaining focus on key pre-market review thresholds) and to consider appropriate weight to both pre-market and post-market review activities. This act also provided increased flexibility in classification of new products, allowing some to be brought to market with less administrative burden than in the past. FDA itself developed administrative practices including a process for expedited review, for real-time review, and for up-front protocol review (so-called pre-IDE reviews) that have helped to foster development of new technology. Cutting edge new technologies in molecular diagnostics have included the Veridex circulating cell counter, the Roche AmpliChip for CYP 450, and the TM Biosciences Cystic Fibrosis multiplex assay.

As a result of this expanded regulatory tool box, FDA review times have decreased, its ability to handle new diagnostic products in innovative manners has increased, and a number of break-through diagnostic products in virtually all areas of laboratory medicine have been introduced in the past five years. The FDA review process is now based on a standardized decision template that is published on the FDA office of in vitro diagnostics (OIVD) web page (www.fda.gov/cdrh/oivd). Access to this information provides transparency in our review process, clarity in decision-making, and helps to maintain parity and an even playing field in FDA work. FDA expects the tools describe above to be invaluable in helping to assure that the agency is a partner and not an obstacle in the development of new biosensors.

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References

Ahn, C.H., Choi, J.W., Beaucage, G., Nevin, J.H., Lee, J.B., Puntambekar, A., Lee, J.Y., 2004. Disposable Smart lab on a chip for point-of-care clinical diagnostics. Proc. IEEE 92, 154–173.

Aina, O.H., Sroka, T.C., Chen, M.L., Lam, K.S., 2002. Therapeutic cancer targeting peptides. Biopolymers 66, 184–199.

Babacan, S., Pivarnik, P., Letcher, S., Rand, A.G., 2000. Evaluation of antibody immobilization methods for piezoelectric biosensor application. Biosens. Bioelectron. 15, 615–621.

Barker, S.L.R., Tarlov, M.J., Canavan, H., Hickman, J.J., Locascio, L.E., 2000. Plastic microfluidic devices modified with polyelectrolyte multilayers. Anal. Chem. 72, 4899–4903.

Barry, M.A., Dower, W.J., Johnston, S.A., 1996. Toward cell-targeting gene therapy vectors: selection of cell-binding peptides from random peptide-presenting phage libraries. Nat. Med. 2, 299–305.

Bast, R.C., Ravdin, P., Hayes, D.F., Bates, S., Fritsche, H., Jessup, J.M., Kemeny, N., Locker, G.Y., Mennel, R.G., Somerfield, M.R., 2001. 2000 Update of recommendations for the use of tumor markers in breast and colorectal cancer: clinical practice guidelines of the American Society of Clinical Oncology. J. Clin. Oncol. 19, 1865–1878.

Becker, H., Locascio, L.E., 2002. Polymer microfluidic devices. Talanta 56, 267–287.

Belizaire, A.K., Tchistiakova, L., St-Pierre, Y., Alakhov, V., 2003. Identification of a murine ICAM-1-specific peptide by subtractive phage library selection on cells. Biochem. Biophys. Res. Commun. 309, 625–630.

Breadmore, M.C., Wolfe, K.A., Arcibal, I.G., Leung, W.K., Dickson, D., Giordano, B.C., Power, M.E., Ferrance, J.P., Feldman, S.H., Norris, P.M., Landers, J.P., 2003. Microchip-based purification of DNA from biological samples. Anal. Chem. 75, 1880–1886.

Brody, E.N., Willis, M.C., Smith, J.D., Jayasena, S., Zichi, D., Gold, L., 1999. The use of aptamers in large arrays for molecular diagnostics. Mol. Diagnosis 4, 381–388.

Brown, K.C., 2000. New approaches for cell-specific targeting: identification of cell-selective peptides from combinatorial libraries. Curr. Opin. Chem. Biol. 4, 16–21.

Buch, J.S., Kimball, C., Rosenberger, F., Highsmith, W.E., DeVoe, D.L., Lee, C.S., 2004. DNA mutation detection in a polymer microfluidic network using temperature gradient gel electrophoresis. Anal. Chem. 76, 874–881.

Burg, M.A., Pasqualini, R., Arap, W., Ruoslahti, E., Stallcup, W.B., 1999. NG2 proteoglycan-binding peptides target tumor neovasculature. Cancer Res. 59, 2869–2874.

Burns, M.A., Johnson, B.N., Brahmasandra, S.N., Handique, K., Webster, J.R., Krishnan, M., Sammarco, T.S., Man, P.M., Jones, D., Heldsinger, D., Mastrangelo, C.H., Burke, D.T., 1998. An integrated nanoliter DNA analysis device. Science 282, 484–487.

Caruso, F., Rodda, E., Furlong, D.F., Niikura, K., Okahata, Y., 1997. Quartz crystal microbalance study of DNA immobilization and hybridization for nucleic acid sensor development. Anal. Chem. 69, 2043–2049.

Cerchia, L., Hamm, J., Libri, D., Tavitian, B., de Franciscis, V., 2002. Nucleic acid aptamers in cancer medicine. FEBS Lett. 528, 12–16.

Chambers, R.S., Johnston, S.A., 2003. High-level generation of polyclonal antibodies by genetic immunization. Nat. Biotechnol. 21, 1088–1092.

Chen, Y.F., Rosenzweig, Z., 2002. Luminescent CdS quantum dots as selective ion probes. Anal. Chem. 74, 5132–5138.

Chou, S.Y., Krauss, P.R., Renstrom, P.J., 1996. Nanoimprint lithography. J. Vac. Sci. Technol. B 14, 4129–4133.

Christel, L.A., Petersen, K., McMillan, W., Northrup, M.A., 1999. Rapid, automated nucleic acid probe assays using silicon microstructures for nucleic acid concentration. J. Biomech. Eng. Trans. ASME 121, 22–27.

DeRoock, I.B., Pennington, M.E., Sroka, T.C., Lam, R.S., Bowden, G.T., Bair, E.L., Cress, A.E., 2001. Synthetic peptides inhibit adhesion of human tumor cells to extracellular matrix proteins. Cancer Res. 61, 3308–3313.

Eaton, B.E., Gold, L., Hicke, B.J., Janjic, N., Jucker, F.M., Sebesta, D.P., Tarasow, T.M., Willis, M.C., Zichi, D.A., 1997. Post-SELEX combinatorial optimization of aptamers. Bioorg. Med. Chem. 5, 1087–1096.

Ekins, R.P., Chu, F.W., 1991. Multianalyte microspot immunoassay—microanalytical compact-disk of the future. Clin. Chem. 37, 1955–1967.

Falconnet, D., Pasqui, D., Park, S., Eckert, R., Schift, H., Gobrecht, J., Barbucci, R., Textor, M., 2004. A novel approach to produce protein nanopatterns by combining nanoimprint lithography and molecular self-assembly. Nano Lett. 4, 1909–1914.

Farah, R.A., Clinchy, B., Herrera, L., Vitetta, E.S., 1998. The development of monoclonal antibodies for the therapy of cancer. Crit. Rev. Eukaryot. Gene Expr. 8, 321–356.

Fawcett, N.C., Craven, R.D., Zhang, P., Evans, J.A., 1998. QCM response to solvated, tethered macromolecules. Anal. Chem. 70, 2876–2880.

Fawcett, N.C., Evans, J.A., Chien, L.C., Flowers, N., 1988. Nucleic-acid hybridization detected by piezoelectric resonance. Anal. Lett. 21, 1099–1114.

Fixe, F., Dufva, M., Telleman, P., Christensen, C.B.V., 2004. Functionalization of poly(methyl methacrylate) (PMMA) as a substrate for DNA microarrays. Nucleic Acids Res., 32.

Ford, S.M., Davies, J., Kar, B., Qi, S.D., McWhorter, S., Soper, S.A., Malek, C.K., 1999. Micromachining in plastics using X-ray lithography for the fabrication of micro-electrophoresis devices. J. Biomech. Eng. Trans. ASME 121, 13–21.

Furtado, L.M., Su, H.B., Thompson, M., Mack, D.P., Hayward, G.L., 1999. Interactions of HIV-1 TAR RNA with Tat-derived peptides discriminated by on-line acoustic wave detector. Anal. Chem. 71, 1167–1175.

Furtado, L.M., Thompson, M., 1998. Hybridization of complementary strand and single base mutated oligonucleotides detected with an on-line acoustic wave sensor. Analyst 123, 1937–1945.

Gambari, R., Borgatti, M., Altomare, L., Manaresi, N., Medoro, G., Romani, A., Tartagni, M., Guerrieri, R., 2003. Applications to cancer research of "lab-on-a-chip" devices based on dielectrophoresis (DEP). Technol. Cancer Res. Treat. 2, 31–39.

Gau, V.J.J., Ho, C.M., McCabe, E.R.B., 2003. Rapid molecular analysis of bacterial infections. Pediatr. Res. 53, 324A.

Gerion, D., Parak, W.J., Williams, S.C., Zanchet, D., Micheel, C.M., Alivisatos, A.P., 2002. Sorting fluorescent nanocrystals with DNA. J. Am. Chem. Soc. 124, 7070–7074.

Gerion, D., Pinaud, F., Williams, S.C., Parak, W.J., Zanchet, D., Weiss, S., Alivisatos, A.P., 2001. Synthesis and properties of biocompatible water-soluble silica-coated CdSe/ZnS semiconductor quantum dots. J. Phys. Chem. B 105, 8861–8871.

Giordano, B.C., Ferrance, J., Swedberg, S., Huhmer, A.F.R., Landers, J.P., 2001. Polymerase chain reaction in polymeric microchips: DNA amplification in less than 240 s. Anal. Biochem. 291, 124–132.

Glennie, M.J., Johnson, P.W.M., 2000. Clinical trials of antibody therapy. Immunol. Today 21, 403–410.

Green, M.C., Murray, J.L., Hortobagyi, G.N., 2000. Monoclonal antibody therapy for solid tumors. Cancer Treat. Rev. 26, 269–286.

Gui, J., Moyana, T., Malcolm, B., Xiang, J., 1996a. Identification of a decapeptide with the binding reactivity for tumor-associated TAG72 antigen from a phage displayed library. Proteins Struct. Funct. Genet. 24, 352–358.

Gui, J., Moyana, T., Xiang, J., 1996b. Selection of a peptide with affinity for the tumor-associated TAG72 antigen from a phage-displayed library. Biochem. Biophys. Res. Commun. 218, 414–419.

Hashimoto, M., Chen, P.C., Mitchell, M.W., Nikitopoulos, D.E., Soper, S.A., Murphy, M.C., 2004. Rapid PCR in a continuous flow device. Lab Chip 4, 638–645.

Hayes, D.F., Bast, R.C., Desch, C.E., Fritsche, H., Kemeny, N.E., Jessup, J.M., Locker, G.Y., MacDonald, J.S., Mennel, R.G., Norton, L., Ravdin, P., Taube, S., Winn, R.J., 1996. Tumor marker utility grading system: a framework to evaluate clinical utility of tumor markers. J. Natl. Cancer Inst. 88, 1456–1466.

Heidari, B., Maximov, I., Sarwe, E.L., Montelius, L., 1999. Large scale nanolithography using nanoimprint lithography. J. Vac. Sci. Technol. B 17, 2961–2964.

Henry, A.C., McCarley, R.L., 2001. Selective deposition of metals on plastics used in the construction of microanalytical devices: photo-directed formation of metal features on PMMA. J. Phys. Chem. B 105, 8755–8761.

Henry, A.C., Tutt, T.J., Galloway, M., Davidson, Y.Y., McWhorter, C.S., Soper, S.A., McCarley, R.L., 2000. Surface modification of poly(methyl methacrylate) used in the fabrication of microanalytical devices. Anal. Chem. 72, 5331–5337.

Huang, Y., Lieber, C.M., 2004. Integrated nanoscale electronics and optoelectronics: exploring nanoscale science and technology through semiconductor nanowires. Pure Appl. Chem. 76, 2051–2068.

Jain, K.K., 2004. Applications of biochips: from diagnostics to personalized medicine. Curr. Opin. Drug Discov. Dev. 7, 285–289.

Johnson, T.J., Ross, D., Gaitan, M., Locascio, L.E., 2001. Laser modification of preformed polymer microchannels: application to reduce band broadening around turns subject to electrokinetic flow. Anal. Chem. 73, 3656–3661.

Jordan, C.E., Corn, R.M., 1997. Surface plasmon resonance imaging measurements of electrostatic biopolymer adsorption onto chemically modified gold surfaces. Anal. Chem. 69, 1449–1456.

Karasseva, N.G., Glinsky, V.V., Chen, N.X., Komatireddy, R., Quinn, T.P., 2002. Identification and characterization of peptides that bind human ErbB-2 selected from a bacteriophage display library. J. Protein Chem. 21, 287–296.

Kim, S., Lim, Y.T., Soltesz, E.G., De Grand, A.M., Lee, J., Nakayama, A., Parker, J.A., Mihaljevic, T., Laurence, R.G., Dor, D.M., Cohn, L.H., Bawendi, M.G., Frangioni, J.V., 2004. Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. Nat. Biotechnol. 22, 93–97.

Kricka, L.J., 2002. Stains, labels and detection strategies for nucleic acids assays. Ann. Clin. Biochem. 39, 114–129.

La Clair, J.J., Burkart, M.D., 2003. Molecular screening on a compact disc. Org. Biomol. Chem. 1, 3244–3249.

Lagally, E.T., Scherer, J.R., Blazej, R.G., Toriello, N.M., Diep, B.A., Ramchandani, M., Sensabaugh, G.F., Riley, L.W., Mathies, R.A., 2004. Integrated portable genetic analysis microsystem for pathogen/infectious disease detection. Anal. Chem. 76, 3162–3170.

Lai, S.Y., Cao, X., Lee, L.J., 2004. A packaging technique for polymer microfluidic platforms. Anal. Chem. 76, 1175–1183.

Leaf, C., 2004. Why we're losing the war on cancer (and how to win it) (Avastin Erbitux, Gleevac.) the new wonder drugs might make you think we're finally beating this dreaded scourage. We're not. Here's how to turn the fight around. Fortune, 76–94.

Lee, H.J., Goodrich, T.T., Corn, R.M., 2001. SPR imaging measurements of 1-D and 2-D DNA microarrays created from microfluidic channels on gold thin films. Anal. Chem. 73, 5525–5531.

Liu, Y., Yu, X., Zhao, R., Shangguan, D.H., Bo, Z.Y., Liu, G.Q., 2003. Real time kinetic analysis of the interaction between immunoglobulin G and histidine using quartz crystal microbalance biosensor in solution. Biosens. Bioelectron. 18, 1419–1427.

Mannelli, I., Minunni, M., Tombelli, S., Mascini, M., 2003. Quartz crystal microbalance (QCM) affinity biosensor for genetically modified organisms (GMOs) detection. Biosens. Bioelectron. 18, 129–140.

Maxwell, D.J., Taylor, J.R., Nie, S.M., 2002. Self-assembled nanoparticle probes for recognition and detection of biomolecules. J. Am. Chem. Soc. 124, 9606–9612.

McGuire, M.J., Samli, K.N., Johnston, S.A., Brown, K.C., 2004a. In vitro selection of a peptide with high selectivity for cardiomyocytes in vivo. J. Mol. Biol. 342, 171–182.

McGuire, M.J., Sykes, K.F., Samli, K.N., Timares, L., Barry, M.A., Stemke-Hale, K., Tagliaferri, F., Logan, M., Jansa, K., Takashima, A., Brown, K.C., Johnston, S.A., 2004b. A library-selected, Langerhans cell-targeting peptide enhances an immune response. DNA Cell Biol. 23, 742–752.

Mikawa, M., Wang, H., Guo, L.L., Liu, R.W., Marik, J., Takada, Y., Lam, K., Lau, D., 2004. Novel peptide ligands for integrin alpha(4)beta(1) overexpressed in cancer cells. Mol. Cancer Ther. 3, 1329–1334.

Minunni, M., Tombelli, S., Scielzi, R., Mannelli, I., Mascini, M., Gaudiano, C., 2003. Detection of beta-thalassemia by a DNA piezoelectric biosensor coupled with polymerase chain reaction. Anal. Chim. Acta 481, 55–64.

Nelson, B.P., Grimsrud, T.E., Liles, M.R., Goodman, R.M., Corn, R.M., 2001. Surface plasmon resonance imaging measurements of DNA and RNA hybridization adsorption onto DNA microarrays. Anal. Chem. 73, 1–7.

Nelson, B.P., Liles, M.R., Frederick, K.B., Corn, R.M., Goodman, R.M., 2002. Label-free detection of 16S ribosomal RNA hybridization on reusable DNA arrays using surface plasmon resonance imaging. Environ. Microbiol. 4, 735–743.

Noerholm, M., Bruus, H., Jakobsen, M.H., Telleman, P., Ramsing, N.B., 2004. Polymer microfluidic chip for online monitoring of microarray hybridizations. Lab Chip 4, 28–37.

O'Sullivan, C.K., Guilbault, G.G., 1999. Commercial quartz crystal microbalances—theory and applications. Biosens. Bioelectron. 14, 663–670.

Oyama, T., Sykes, K.F., Samli, K.N., Minna, J.D., Johnston, S.A., Brown, K.C., 2003. Isolation of lung tumor specific peptides from a random peptide library: generation of diagnostic and cell-targeting reagents. Cancer Lett. 202, 219–230.

Pal, D., Venkataraman, V., 2002. A portable battery-operated chip thermocycler based on induction heating. Sens. Actuators A-Phys. 102, 151–156.

Pasqualini, R., Koivunen, E., Ruoslahti, E., 1997. alpha v Integrins as receptors for tumor targeting by circulating ligands. Nat. Biotechnol. 15, 542–546.

Pietersz, G.A., McKenzie, I.F.C., 1992. Antibody conjugates for the treatment of cancer. Immunol. Rev. 129, 57–80.

Roper, M.G., Easley, C.J., Landers, J.P., 2005. Advances in polymerase chain reaction on microfluidic chips. Anal. Chem. 77, 3887–3893.

Rossier, J., Reymond, F., Michel, P.E., 2002. Polymer microfluidic chips for electrochemical and biochemical analyses. Electrophoresis 23, 858–867.

Rudert, F., 2000. Genomics and proteomics tools for the clinic. Curr. Opin. Mol. Ther. 2, 633–642.

Schatzlein, A.G., Rutherford, C., Corrihons, F., Moore, B.D., 2001. Phage derived peptides for targeting of doxorubicin conjugates to solid tumours. J. Control. Release 74, 357–362.

Skelley, A.M., Scherer, J.R., Aubrey, A.D., Grover, W.H., Ivester, R.H.C., Ehrenfreund, P., Grunthaner, F.J., Bada, J.L., Mathies, R.A., 2005. Development and evaluation of a microdevice for amino acid biomarker detection and analysis on Mars. Proc. Natl. Acad. Sci. U.S.A. 102, 1041–1046.

Smith, E.A., Corn, R.M., 2003. Surface plasmon resonance imaging as a tool to monitor biomolecular interactions in an array based format. Appl. Spectrosc. 57, 320A–332A.

Soper, S.A., Ford, S.M., Qi, S., McCarley, R.L., Kelly, K., Murphy, M.C., 2000. Polymeric microelectromechanical systems. Anal. Chem. 72, 642A–651A.

Su, H.B., Chong, S., Thompson, M., 1997. Kinetics of hybridization of interfacial RNA homopolymer studied by thickness-shear mode acoustic wave sensor. Biosens. Bioelectron. 12, 161–173.

Sun, C.P., Liao, J.C., Zhang, Y.H., Gau, V., Mastali, M., Babbitt, J.T., Grundfest, W.S., Churchill, B.M., McCabe, E.R.B., Haake, D.A., 2005. Rapid, species-specific detection of uropathogen 16S rDNA and rRNA at ambient temperature by dot–blot hybridization and an electrochemical sensor array. Mol. Genet. Metab. 84, 90–99.

Tan, H., Gilbertson, A., Chou, S.Y., 1998. Roller nanoimprint lithography. J. Vac. Sci. Technol. B 16, 3926–3928.

Thiel, A.J., Frutos, A.G., Jordan, C.E., Corn, R.M., Smith, L.M., 1997. In situ surface plasmon resonance imaging detection of DNA hybridization to oligonucleotide arrays on gold surfaces. Anal. Chem. 69, 4948–4956.

Tombelli, S., Mascini, M., Turner, A.P.F., 2002. Improved procedures for immobilisation of oligonucleotides on gold-coated piezoelectric quartz crystals. Biosens. Bioelectron. 17, 929–936.

Ulrich, H., Alves, M.J.M., Colli, W., 2001. RNA and DNA aptamers as potential tools to prevent cell adhesion in disease. Braz. J. Med. Biol. Res. 34, 295–300.

Unger, M.A., Chou, H.P., Thorsen, T., Scherer, A., Quake, S.R., 2000. Monolithic microfabricated valves and pumps by multilayer soft lithography. Science 288, 113–116.

Varma, M.M., Inerowicz, H.D., Regnier, F.E., Nolte, D.D., 2004a. High-speed label-free detection by spinning-disk micro-interferometry. Biosens. Bioelectron. 19, 1371–1376.

Varma, M.M., Nolte, D.D., Inerowicz, H.D., Regnier, F.E., 2004b. Spinning-disk self-referencing interferometry of antigen–antibody recognition. Opt. Lett. 29, 950–952.

Wang, J., 2000. From DNA biosensors to gene chips. Nucleic Acids Res. 28, 3011–3016.

Wang, W.U., Chen, C., Lin, K.H., Fang, Y., Lieber, C.M., 2005. Label-free detection of small-molecule-protein interactions by using nanowire nanosensors. Proc. Natl. Acad. Sci. U.S.A. 102, 3208–3212.

Wang, Y., Vaidya, B., Farquar, H.D., Stryjewski, W., Hammer, R.P., McCarley, R.L., Soper, S.A., Cheng, Y.W., Barany, F., 2003. Microarrays assembled in microfluidic chips fabricated from poly(methyl methacrylate) for the detection of low-abundant DNA mutations. Anal. Chem. 75, 1130–1140.

Willner, I., Patolsky, F., Weizmann, Y., Willner, B., 2002. Amplified detection of single-base mismatches in DNA using micro gravimetric quartz-crystal-microbalance transduction. Talanta 56, 847–856.

Wolfe, K.A., Breadmore, M.C., Ferrance, J.P., Power, M.E., Conroy, J.F., Norris, P.M., Landers, J.P., 2002. Toward a microchip-based solid-phase extraction method for isolation of nucleic acids. Electrophoresis 23, 727–733.

Woolley, A.T., Guillemette, C., Cheung, C.L., Housman, D.E., Lieber, C.M., 2000. Direct haplotyping of kilobase-size DNA using carbon nanotube probes. Nat. Biotechnol. 18, 760–763.

Zhou, X.C., Huang, L.Q., Li, S.F.Y., 2001. Microgravimetric DNA sensor based on quartz crystal microbalance: comparison of oligonucleotide immobilization methods and the application in genetic diagnosis. Biosens. Bioelectron. 16, 85–95.

Zhou, X.D., Liu, L.J., Hu, M., Wang, L.L., Hu, J.M., 2002. Detection of hepatitis B virus by piezoelectric biosensor. J. Pharm. Biomed. Anal. 27, 341–345.