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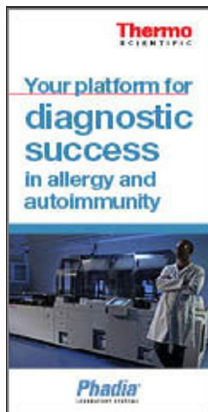
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Clinical applications of a programmable nano-bio-chip

Analytical systems that can be adapted rapidly to be able to detect new cellular and circulating biomarkers of disease as they emerge from basic research are clearly of great benefit in the clinical environment. In the development of such systems, two aspects are particularly important, namely the creation of customised analyte panels specific for different disease states and the ability to make measurements with a high degree of analytical integrity at the point-of-care. Here we describe clinical results obtained with a nano-bio-chip and its application in HIV monitoring, chest pain diagnosis and gynaecological cancer screening.

by Dr J. V. Jokerst, Dr B. D. Bhagwandin, Dr J. W. Jacobson & Prof. J. T. McDevitt

Obtaining accurate measurements of meaningful biomedical analytes makes the clinical hospital laboratory a key part of the overall healthcare infrastructure by providing information on circulating levels of cellular, proteomic and genetic biomarkers. From the results of such clinical tests, critical patient care decisions can be made. Unfortunately, traditional laboratory technologies cannot keep pace with the ever-expanding number and complexity of clinically relevant biomarkers and sample types [1, 2]. Current clinical instrumentation is for the most part optimised for specific existing biomarkers and newly identified bioanalytes cannot easily be incorporated. This missing link in the analytical infrastructure is a significant barrier to the incorporation of new biomarker signatures derived from genomic and proteomic studies. While these biomarkers can yield significant academic insight into the underlying features of disease states, the fact that only about one biomarker per year is approved in the US by the FDA for clinical practice effectively decouples this new bioinformatics-derived information from modern clinical practice. Furthermore, the expense of constantly replacing or upgrading existing equipment rather than using easily reprogrammed designs contributes significantly to the rapid increase in national healthcare budgets [3].

The need for inexpensive, modular, rapid and customisable instrumentation is currently the driver for a broad group of scientists, clinicians, technologists and engineers in the development of new technologies that could be used in such multiclass, multiplexed detection systems [4]. These adaptable biomedical devices increasingly command quality results. The acronym COMMAND QUALS summarises the desired attributes of coherently designed biosensors. Thus, the objective of integrated micro-medical approaches is the creation of devices that are: Cheap, Obvious, Miniaturised, Multiplexed, Automated, Non-perishable, Dependable, Quick, Unobtrusive with regard to specimens and sampling procedures, Adaptable to a variety of analytes, Limited in reagent and sample volume requirements, and Self-contained. Although no single approach has emerged to satisfy all of these requirements, the programmable nano-bio-chip (NBC) is a promising technology with the potential to condense many elements of the clinical laboratory (haematology, pathology, microbiology, chemistry, and serology) onto a single, simple microchip sensor system. Furthermore, due to its reduced size, the NBC has the capacity to perform such analyses at the point-of-care (e.g. in the ambulance, at the bedside, in the physician's office, or in a remote location) and thus has the potential to bridge the gap between diagnostic information, providers, and patients, so enabling co-localised diagnostics, therapeutic decisions and actions [5].

The nano-bio-chip (NBC)

The NBC is flexible and comes in two basic classes namely for use with either soluble analytes or bioparticles, e.g. cells and spores. The NBC systems use a size-tunable nano-net within either agarose microspheres or a polymer membrane and use a fluorescent transduction signal from nanoparticles

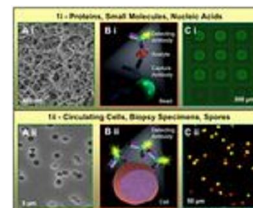


Figure 1. The NBC approach to clinical measurements uses advances in the fields of nanotechnology, bio medicine and microfluidics. In panel A, scanning electron microscope images show the core components. Ai is the agarose bead and the nano-meter-scale network of fibres that serve to preconcentrate and localise biomolecules from the bulk solution, while Aii is the polycarbonate membrane that is used for bioparticle capture. Analyte-specific signal comes from a fluorescently labelled detecting antibody either on the bead or cell surface (Panels Bi and Bii respectively). (Note that antibodies are not drawn to scale with respect to either the 280 μm bead or $\sim 10 \mu\text{m}$ cell.) Bead arrays are then placed within a metallic wafer (Panel Ci) to secure them in the microfluidic flow chamber. Alternatively, fluorescently labelled cells populate the membrane filter for interpretation by digital processing software (Panel Cii).

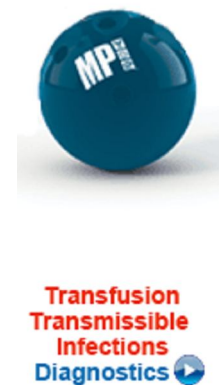


Figure 2. A) Schematic diagram of NBC analyser that is currently in development at LabNow. Assays completed with NBC are performed with the compact, toaster-sized analyser. The biochemical reactions detailed in Figure 1 are all performed within the compact labcard (bottom right Panel A). The standalone analyser core (Panel B) contains LED excitation sources, actuators for fluid handling, and a digital camera for image capture. The complete device and labcard (C) create an integrated instrumentation and assay system (Panel C).

(nano) to isolate and quantify biologically important analytes (bio) from complex matrices within a closed, miniaturised system (chip). As a multi-functional clinical tool, the NBC has demonstrated applications in measuring numerous sample types and analyte subsets encountered in the clinical lab. These include T-lymphocytes, small molecules, polypeptides, ions, sugars, biological cofactors, glycoproteins, nucleic acid fragments, biopsy specimens and spores [6-13]. The molecular level detail of the NBC approach is shown in Figure 1 with emphasis on the main analyte capture elements and the signal generation step. For the analysis of soluble material such as genomic and proteomic biomarkers, a bead-based approach is used [Figure 1, Panel Ai]. Here, 280 µm diameter beads behave as miniaturised sponges that deplete and concentrate target molecule from the specimen with the aid of monoclonal antibodies that are covalently linked to the network of agarose strands. Alternatively, for particulate-based analyses, e.g. cells and spores [Figure Aii] a membrane-based approach is used and size-selective analysis with fluorescence labelling is used to identify analytes of interest. Both the bead- and membrane-based approaches use fluorescent reporter molecules for signal generation and a self-contained, microfluidic flow chamber to control the flow behaviour of the specimen and reagents, and to contain waste.

The integrated design of the NBC contains all elements required for a complete assay, condensed into a credit card-sized disposable cartridge, the labcard. Liquid reagents are stored in blister packs while fluorescently tagged bio-recognition moieties are preserved in solid form. Channels designed for mixing and fluid flow permeate this architecture and, depending on the design, manipulations of the fluidic cartridges can bring about reagent reconstitution and dispersal through the labcard. Cards are disposable and designed to process one patient sample. Specimen collection is either by venipuncture or finger-stick for blood samples or simple expectoration for saliva. The sample is maintained briefly in a capillary tube before the introduction, via capillary action, into the compact NBC. The labcard is then inserted into the analyser where bursting of the fluid-containing blister packs complete the assay. An illustration of the analyser that interfaces with the disposable fluidic cards is shown in Figure 2. This universal instrumentation platform has a footprint of about the size of a toaster with a cost about one-fifth of the current macroscopic instruments [14]. The assay outputs are displayed on a built-in screen. The device can be powered from the mains or from batteries, with a battery life of several hours. Optical signal capture occurs via a magnifying objective lens; the downstream processing software, read-out display, memory for up to 50,000 patient histories, and USB/Ethernet/wireless communication features complete the device. The total weight is 6 Kg, which makes the system suitable for measurements at the point-of-care (POC), e.g. at the bedside, in the ER, ambulance or in resource-poor settings, etc.

By using the nano-, bio- and chip-based properties, impressive analytical performance can be achieved as seen in the study of the acute phase inflammatory marker, C-reactive protein [Table 1]. It can be seen that the microchip approach yields assay variance values comparable to those from mature macroscopic instruments. In fact, the bead-based NBC has a lower limit of detection, making it potentially suitable for the first time for salivary CRP measurements.

In addition to the broad range of analytes, the NBC approach has been adapted to a wide range of sample types and it has been found that different biofluids can be analysed with the same core technology. By maintaining the same fluid and light handling equipment across all of these assays, development times and costs are minimised and universality can be maintained. Assays designed in a strategic way from the same fundamental building blocks are integrated into the NBC, which now has a catalogue of optimised assays for easy expansion into customised applications and panels. Custom bead combinations, each with a unique molecular-level code, vary according to disease state. In cardiovascular medicine, for example, one could prepare different chip panels for the prevention visit (atherosclerosis, arteriosclerosis), the emergency department (chest pain aetiology), and congestive heart failure (cardiologist specialist). These different panels are created with a similar process flow that is inspired in part by the modular manufacturing methods used in the electronics industry. By simply removing certain bead types and adding others, an entirely different panel can be created, while the core microchip and fluid flow of the chemical processor is retained. The analyser hardware, which includes the mechanical, software and optical interfaces remains constant from one test to another. A bar code reader built-in to the analyser is used to recognise any new, customised NBC that may have been developed, together with any associated specific image analysis routines.

Diverse analyses, one system

Now that the broad range of analyte capabilities of the new NBC system has been demonstrated, it is increasingly being applied to a number of key clinical applications. For example, a common application is the analysis of T lymphocytes in HIV patients, such as the determination of the CD3+ CD4+ cell counts that are required to determine immune system status and efficacy of antiretroviral treatment. Using the simple, yet elegant cell capture mechanism, the polycarbonate, track-etched membrane retains the larger and more rigid lymphocytes while allowing blood matrix components including plasma, platelets, and erythrocytes to pass to a waste reservoir. In pilot studies (n = 200), the NBC results correlated nicely (R2 = 0.94) with those from flow cytometry; baseline separation between CD4 positive lymphocytes and monocytes was achieved. The data show that the device can produce reliable results from finger-prick sized samples (30 µL) in under 20 minutes [5, 8]. The technique is also suitable for paediatric testing, e.g. for total white blood cell counts and differential testing.

In addition to monitoring cellular analytes in blood, the NBC may be used to measure soluble blood components such as proteins. Saliva is emerging as a body fluid that has a number of features which make it highly compatible with diagnostic testing. First, an increasing body of evidence suggests that saliva serves as a "mirror of the body" and contains biomarkers that

Assay	Substrate	Assay Range	Limit of Detection	Time (min)	Time (sec)
CRP	BSA	0.01-100 µg/L	0.01 µg/L	10	1-100
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CRP	BSA	0.01-100 µg/L	0.01 µg/L	10	1-100

Table 1. The analytical performance of the NBC device is very competitive with existing technologies including ELISA and typical high throughput clinical analysers as shown here for a C-reactive protein assay. In addition to very low limits of detection (essential for saliva), the NBC has turnaround times consistent with emergency events at the point-of-care. Standard techniques shown above include immunonephelometric (IN), immunoturbidimetric (IT), and immunoluminometric (IL). The table summarises assays now in development, but not yet commercially available.

Assay	Substrate	Assay Range	Limit of Detection	Time (min)	Time (sec)
CRP	BSA	0.01-100 µg/L	0.01 µg/L	10	1-100
CRP	BSA	0.01-100 µg/L	0.01 µg/L	10	1-100
CRP	BSA	0.01-100 µg/L	0.01 µg/L	10	1-100
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CRP	BSA	0.01-100 µg/L	0.01 µg/L	10	1-100
CRP	BSA	0.01-100 µg/L	0.01 µg/L	10	1-100
CRP	BSA	0.01-100 µg/L	0.01 µg/L	10	1-100
CRP	BSA	0.01-100 µg/L	0.01 µg/L	10	1-100
CRP	BSA	0.01-100 µg/L	0.01 µg/L	10	1-100

Table 2. The NBC has documented capabilities for measuring a broad range of analyte and sample types as measured with lab-based prototypes.

offer important information on both oral and systemic disease, albeit at concentrations markedly different from those in serum [15, 16]. New salivary biomarkers continue to be evaluated and reported. These nascent molecules can easily be included in the NBC. In addition, as a constantly regenerated fluid, saliva offers a better 'physiological snapshot' than serum and requires little post-collection processing, unlike blood. More important is the ease with which saliva samples can be collected. Expectoration by the patient or collection by the technician is straightforward, safer than venipuncture and results in much less dangerous medical waste. For patients, the non-invasive collection method of oral fluid sampling reduces anxiety and discomfort.

We have recently demonstrated that the NBC can detect certain molecules in saliva which could be used for screening purposes. In one study on carcinoembryonic antigen (CEA), the limits of detection of the NBC approach were nearly two orders of magnitude lower than those of ELISA [7]. In addition, it was shown that NBC determination of CEA correlated well with standard analytical methods ($R^2 = 0.94$ and $R^2 = 0.95$ for saliva and serum, respectively). In pilot studies of patients with acute myocardial infarction (AMI), the cardiac-specific markers myoglobin (MYO), myeloperoxidase (MPO), troponin I (TnI), brain natriuretic protein (BNP), creatinine phosphokinase myocardial band (CK-MB), and C-reactive protein (CRP) were analysed on the NBC [17]. Serum and unstimulated whole saliva (UWS) samples from an AMI-positive population ($n = 41$) and control group ($n = 21$) were analysed for these cardiac biomarkers by both the NBC and standard reference methods. It was shown that serum levels of the biomarkers were significantly raised in the disease group compared to the AMI-negative group. Data interpretation methods such as logistic regression, area under curve (AUC) and receiver-operator characteristic (ROC) analysis were used to evaluate the diagnostic impact that certain combinations of these biomarkers could have. In saliva samples analysed on the NBC, the results of CRP, MYO, and MPO yielded AUC values of 0.85 ($p < 0.0001$); when electrocardiogram (ECG) was added, the AUC values increased to 0.96. In serum, the results of panels of BNP, TnI, MYO and CK-MB had AUC values of 0.98, far superior to the approximately 0.6 AUC values typical of ECG analysis alone. By using the NBC to analyse saliva, it is easy to imagine such analyses being carried out in an ambulance as opposed to a centralised hospital lab. The range of tests that can be carried out on saliva using the NBC is continuing to expand [Table 2].

Prospects

The trend towards compact miniaturised, modular biomedical devices looks set to continue in coming decades. For the scientific community, an important goal is now to design such systems in a way convenient to patient, user and physician, while maintaining analytical quality. NBC technology, with its à la carte assay programming, fast turnaround times, highly competitive analytical performance and a compact, portable design could provide robust solutions to such challenges. Table 2 shows the diverse array of strategic panels that can be created with the same miniaturised flow components. While additional studies are certainly needed to assess more fully the performance of the NBC, work is currently being carried out to transform the discoveries into a form suitable for practical use. The use of the NBC in settings such as the ambulance, operating room and physician's office is being actively explored and has the potential to both decrease costs and improve patient outcomes.

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