Programmable Bio-Nano-Chip System:

Flexible Platform for Bioscience and Clinical Measurements

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Advances in lab-on-a-chip systems have strong potential for detection of a wide range of analytes with reduced sample and reagent volume, lower costs and shorter analysis times. The completion of high-fidelity multiplexed and multiclass assays remains a huge challenge for the medical microdevice field, as it struggles to achieve and expand upon results that are achieved now routinely in remote laboratory settings with new technologies that can complete similar studies at the point-of-care.

This paper features a public-private collaboration that has generated powerful new programmable bio-nano-chip cartridges suitable for both research and clinical usage. A case study here summarized features prototype microfluidic cartridges from the McDevitt Research Group (Rice University) that have been adapted to include MiniFAB's design for manufacturing expertise as well as unique filter and membrane components as provided by Porex.

The output of this fruitful collaboration is a customizable single use, disposable cartridge that can be manufactured in high volumes and at significantly lower cost compared to prior prototypes. The cartridge has been designed to service both multiplexed and multi-class testing. The flexible platform is suitable for general chemistries, protein, antibody, DNA/RNA, cell, bacteria and spore application areas. This platform system, now on a path towards high volume manufacture, has potential to outperform all previous prototypes in terms of analytical performance, ease of use, reproducibility and cost of production. More importantly, this microfluidic cartridge has potential to serve as a standard test ensemble and is poised to accelerate the ongoing six major clinical trials in the areas of oral cancer, prostate cancer, ovarian cancer, cardiac heart disease and illicit drug detection.

Microfluidics Card Module

The POC analysis solution here described, the p-BNC, synergizes components and achievements from nanotechnology, molecular diagnostics, microfluidics, and microelectronics to create a powerful new measurement approach in a small device footprint. The p-BNC ensemble employs a size-tunable network of nanometer-scale fibers (“nano-net”) within agarose microspheres or polymer membrane and a fluorescent signal arising from nanoparticles (nano) to isolate and quantify biologically important analytes (bio) from complex matrices within a closed, miniaturized system (chip).

The p-BNC lab-card was developed through two major steps involving a systematic study of subcomponents developed through a laminate-based rapid prototype procedure followed by a translation of the optimized configuration into injection molded plastic structures. Figure 1 depicts the internal microfluidic circuitry of the p-BNC assay cartridge as optimized from the rapid prototype efforts.

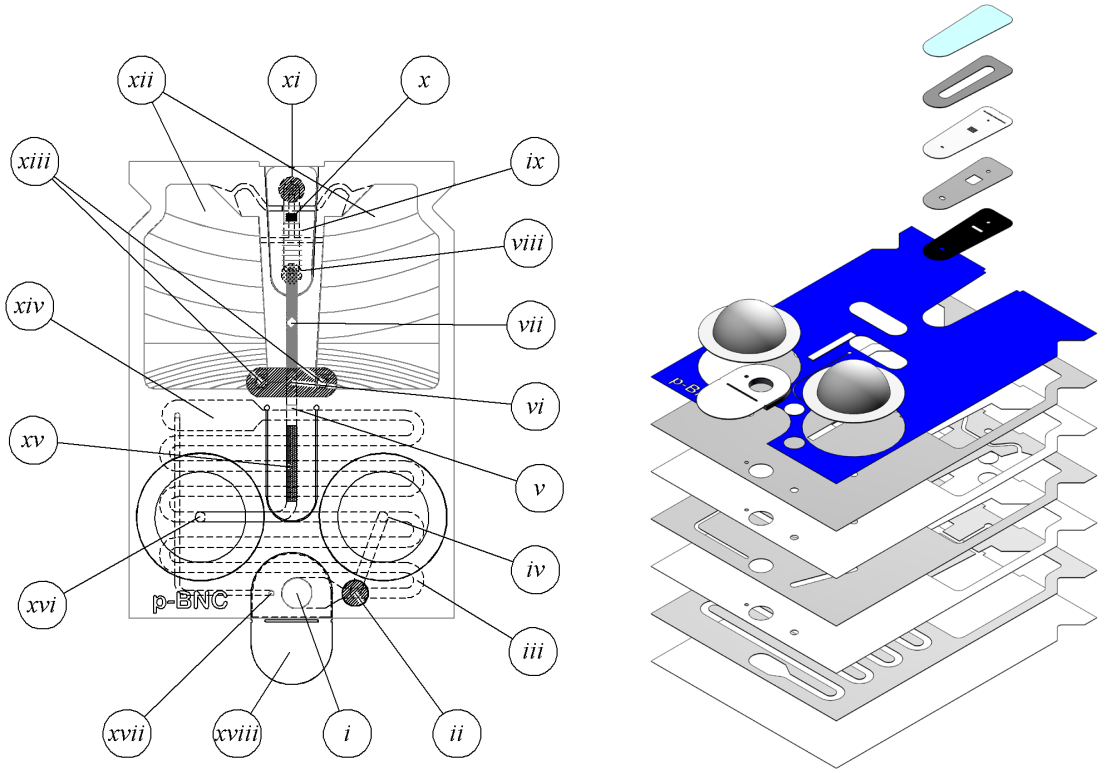


Figure 1: The piping diagram above shows the features of the p-BNC’s microfluidic network. The sample metering channel features (i) a port for sample input with (xviii) a flip cap, (ii) bubble trap, (iii) 100µL capacity metered sample loop and (xiv) a passive valve (v) and a 20µL sample overflow chamber leading to (xvii) external vent. Right-hand fluid input port (iv) intersects sample loop at a strategic location to evacuate metered portion of sample toward bead sensors.

The left-hand fluid input hole (xviii) is connected to the solid-state reagent storage chamber (xv) followed by a (vi) bubble trap. Both the sample metering channel and reagent preparation channel confluence at the distal bubble trap, which forms a junction column with a single output. The common channel, on the same plane as the sample loop, features two sets of staggered herringbone mixers (vii) and an inline track-etch membrane (viii).

The mixer channel provides access to the bead array (x) followed by a large capacity waste chamber reservoir (xii). Grooves patterned on the top and bottom of waste reservoir help direct fill to downstream vents (xiii) covered with selectively permeable vent membranes for secure waste containment.

Up to this juncture, there are many examples of microarrays and several emerging examples of lab-on-a-chip systems. The latter to date have almost exclusively been dedicated to single analyte class testing. Single function devices like cDNA microarrays and blood glucometers represent essential places to start, but for many real-world clinical biosensing scenarios, it is most desirable to complete testing across analyte classes and acquire strategic disease specific panels. Thus, the lack of a standard modular testing platform for multiplexed and multiclass analyte testing is a major barrier for the field, especially for lab-on-a-chip structures that are much more sophisticated to develop in the context of fully integrated test modalities.

With this perspective in mind, the McDevitt group has sustained concerted efforts to move multiplexed and multiclass chem- and bio-sensing onto a single modular lab-on-a-chip platform. Likewise, this key focus differentiates the p-BNC from the analytical schema developed previously. Unlike the approaches for the majority of microfluidics, biochip, and lab-on-a-chip paradigms in which one 'chip' is created specific to a type of cell/protein/oligo, the p-BNC has a broad portfolio of analytes that are measured with the same compact system. Assays for cells, proteins, and nucleic acids can be completed within the universal compact, disposable reaction labcard which houses both beads for protein measurements and membranes for cell differentiation and counting. Targeted panels are created easily through the inclusion of modular reagent packages, bead capture elements, and size-tuned cell collection ensembles. Another important distinction is the engineered micro-environment for analyte capture. While many microfluidic approaches use planar arrays, the p-BNC now in development employs high surface area three-dimensional beads that serve to efficiently concentrate various analytes from the biofluid specimen.

In addition to the expanded menu, another key area of focus in the area of biosensors is a decrease in component size toward miniaturized designs—for clinical systems, these efforts can lead to medical results at the point-of-care (POC), i.e. bedside, ambulance, and other remote locations. In spite of the remarkable progress made toward POC clinical assay systems, very few complete working prototypes have emerged. Although promising starts have been observed with microfluidic lab-on-a-chip approaches, and important goals defined with the micro total analysis system (μTAS) paradigm, the broad-scale release of workable devices based on these premises is largely incomplete. These structures still require macroscopic laboratory-based infrastructure. While their analysis core is substantially smaller than their bench-top alternatives, the network of support structures required for sample processing, data collection, and reagent handling imply that these platforms are best described as 'chips in a lab' rather than true labs-on-a-chip. The absence of a standard, universal, and modular analysis technology motivates this work toward the development of systems amenable to rapid prototyping with easy inclusion of newly validated biomarkers.

The final major challenge area for lab-on-a-chip systems has been in the area of clinical validation. Very few medical microdevice systems have been validated in major clinical trials. Multiplexed and multiclass validation studies are sorely lacking as has been the identification of the 'killer app' for the area of microfluidic sensor systems. The three major deficiencies for LOC sensors are addressed below in the context of the description of a flexible and scalable lab-on-a-chip test platform.

Design For Manufacture (DFM)

Following a thorough understanding of the product requirements and the challenges highlighted by testing of the above-described laminate prototype, the starting point for DFM was to reduce the bill of materials (BOM) as far as possible. Initial targets in this process were the replacement of the multilayer laminate structure with a single injection moulded component (integrating the sample metering, reagent dispense, bubble traps, reagent mixers, detection chamber and waste chambers), and reduction in the number of individual components used for membranes and filters. Material selection was also a priority in order to select materials that were suitable for both processing and the application. Selection of Porex materials was a key consideration during this activity in order to ensure high quality materials, compliant with use in the healthcare market, and that were easy to handle during manufacture. The industrial design of the cartridge was also addressed to create a device that more closely resembled the intended final product. One of MiniFAB's preferred design solutions is to add a shell to the disposable, where the shell performs several important functions:

- Improved aesthetics of the device that can be customised, for example by changing the color
- Provides sufficient surface area for adding labelling
- Provides guides and features to assist in handling the cartridge and loading into the instrument
- Protection for critical components/features of the cartridge such as; the reagent blisters and detection chamber/window, Porex membrane materials
- Protection of the bottom surface of the cartridge to prevent contamination during the work flow
- Adds a textured gripping surface for easy handling of the cartridge

The prototype method of sealing the specimen within the cartridge was by a use of an adhesive tape. This was replaced by a cap component that provided a far more suitable interface between the user and the cartridge.

With a design for the disposable cartridge well advanced the manufacturing process flow can be finalised. The manufacturing steps are typical for MiniFAB's approach to volume manufacturing and can be employed from early prototype builds through to volume production. Based on prior experience, the tolerance stack between the manufacturing of the sub-components and assembly of the final device can determined and the design of the cartridge and sub components adjusted to ensure success. The goal of this activity is to maximise the allowable tolerance within the device which, in turn, can have very beneficial consequences on the cost of manufacturing.

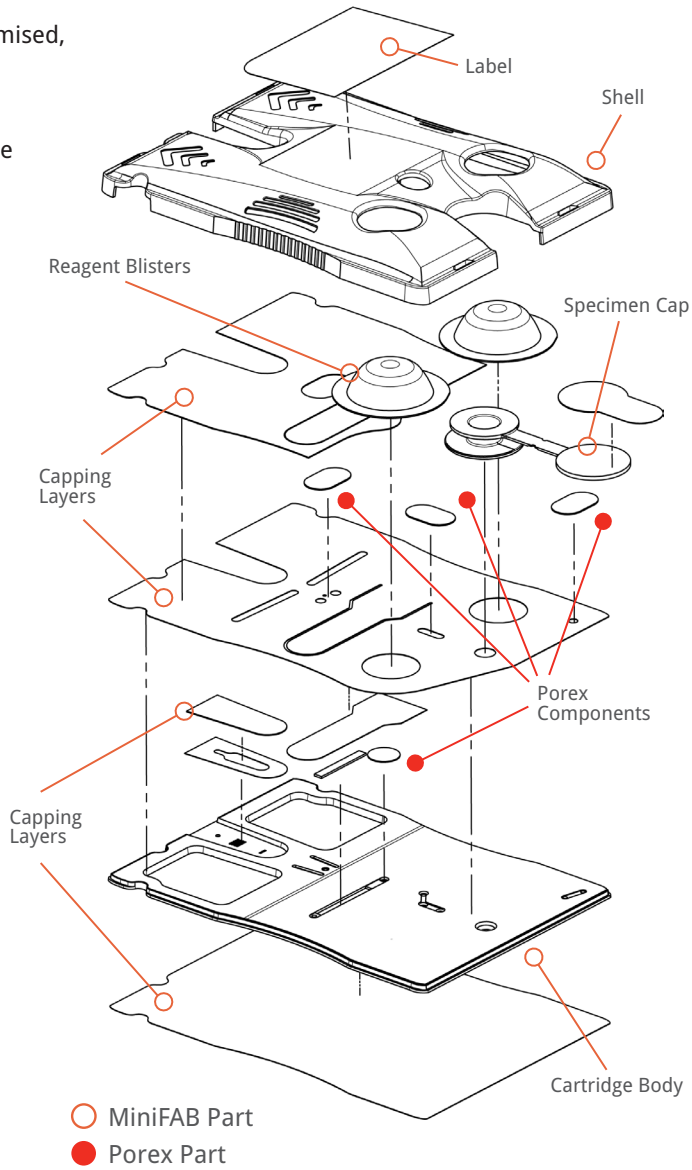


Figure 2: The updated cartridge design following design for manufacture.

Manufactured Cartridges

Following approval of the new cartridge design injection moulding tooling was commissioned for the cartridge base, shell and cap. Porex membrane materials were cut by laser ablation, ahead of transfer to a die cut process. Laminate structures were die cut and simple jigs and fixtures created to assist in the assembly of the cartridges. Porex components and reagent blisters were added to the cartridge in a simple pick and place process. The processes established thus far are consistent with the build of thousands of cartridges and can be scaled to high volumes by automation of these processes using established technology.

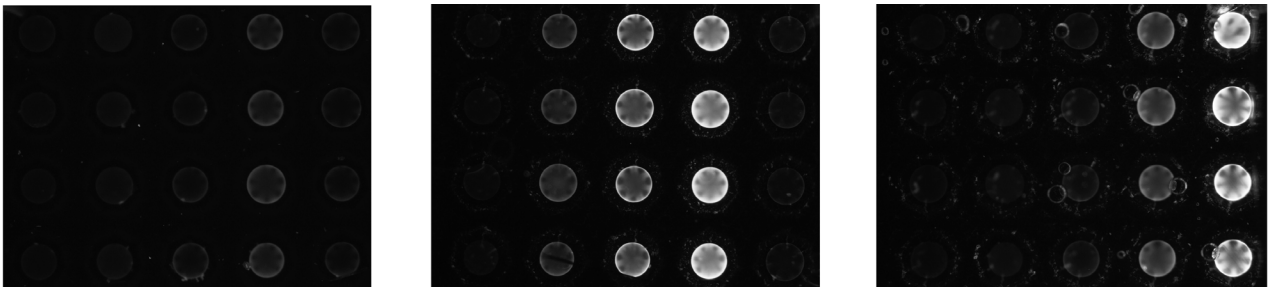
Testing of Fluidic Cartridges

Using the p-BNC concept here described the McDevitt lab has over the past 4 years launched 6 major clinical trials with more than 5,000 patients at 10 clinical sites. At the time of this writing, the initial components for the DFM disposables have just been completed and the initial testing with real-world clinical samples has been initiated. Figure 3 includes concept images for the various colored cartridges that are envisioned for future clinical use. All past efforts have been completed using hand-built laminate structures.

Likewise, the availability of injection molded disposables in this area has potential to accelerate significantly the clinical trials that target major diseases in the areas of cardiac heart disease, oral cancer, ovarian cancer, prostate cancer and drugs of abuse. These on going clinical trial efforts are unique in their capacity to validate the new multi-sensor chip-based platform. Through these efforts, microchips are being customized for disease categories that afflict major populations globally.

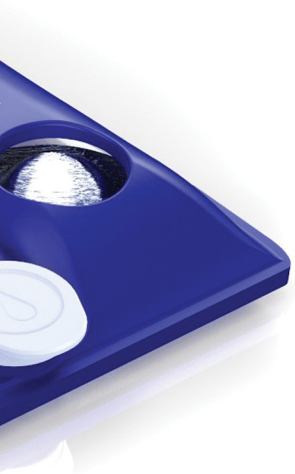
Figure 3: A family of diagnostic devices color-coded for a range of applications.

Key to future successful implementation of these p-BNC tests is the fact that they meet the analytical performance requirements, as dictated by the pathophysiological levels of the various biomarkers for healthy vs. diseased. In order for these chip-based tests to have clinical relevance, they must not only respond on a timeframe consistent with point of care usage, but they must meet or exceed the analytical, and, thereby, clinical performance of the gold standards or reference methods, that are for the most part limited to the laboratory setting (see Table 1). With this in mind, a significant amount of effort has now been devoted to the determination of the analytical performance of key biomarkers using these bead sensor systems. These measurements are completed using human clinical samples and thus move above and beyond the common starting place of purified antigens within buffer solutions.



A B C

Figure 4 displays images obtained for three different types of chest pain patients as recruited by Baylor College of Medicine as part of a 1050 patient trial. Panel A provides a representative view obtained for a false alarm patient. Here most of the bead types are silent. Panel B shows a typical heart attack event. For this patient numerous bead types including c-Tn-I, CK-MB, and myoglobin light up strongly. Whereas panel C shows the characteristics for a heart failure patient where nT-pro-BNP and myoglobin yield dominant responses.



Collectively these images reveal a strong capacity to complete differential diagnosis within the context of chest pain patients. Panel sizes and bead sensors are easily swapped to serve the needs of the diagnostic application. Noted for each array there is four fold redundancy of the bead sensors per analyte, which contributes to accurate and precise measurements.

Biomarker	Clinical Use	Range [a] (ng/mL)	LOD [a] (ng/mL)	Method
C-reactive protein	AMI, Risk Assessment	0.1–10,000	0.1	Theoretical
Soluble CD40 ligand	Cardiac Risk Assessment	0.1–1,000	0.1	Practical
Monocyte chemoattractant protein-1	Cardiac Risk Assessment	0.001–20	0.001	Practical
Myeloperoxidase	Cardiac Risk Assessment	0.05–500	0.05	Practical
Myeloperoxidase (multiplexed)	Cardiac Risk Assessment	1.2–500	1.2	Theoretical
Interleukin-1beta	Cardiac Risk Assessment	0.001–1	0.001	Practical
Interleukin-6	Cardiac Risk Assessment	0.001–1	0.001	Practical
Tumor necrosis factor-alpha	Cardiac Risk Assessment	0.01–10	0.01	Practical
Cardiac troponin I	AMI Diagnosis	0.05–50	0.05	Theoretical
Myoglobin	AMI Diagnosis	0.1–1,000	0.1	Theoretical
CK-MB	AMI Diagnosis	1.7–50	1.7	Theoretical
Apolipoprotein A1	Risk for recurrence/Prognosis	1–1,000	1	Practical
Apolipoprotein B	Risk for recurrence/Prognosis	1–1,000	1	Practical
Brain natriuretic peptide	Congestive Heart Failure	0.05–10	0.05	Theoretical
N-Terminal proBNP	Congestive Heart Failure	0.1–500	0.1	Theoretical
Transferrin	Blood contamination in saliva	0.05–10,000 [b]	0.05 [b]	Theoretical
Carcinoembryonic antigen	Ovarian Cancer	0.1–100	0.02	Theoretical
Cancer antigen 125	Ovarian Cancer	1–400 [c]	1 [c]	Theoretical
Prostate-specific antigen	Prostate Cancer	0.1–100	0.1	Theoretical
Free prostate-specific antigen	Prostate Cancer	0.1–100	0.1	Theoretical
Complexed prostate-specific antigen	Prostate Cancer	0.63–100	0.63	Theoretical
Cocaine	Road Side Drug Testing	1.3–10,000	1.3	Practical
Morphine	Road Side Drug Testing	0.46–1,000	0.46	Theoretical
Diazepam	Road Side Drug Testing	0.14–1,000	0.14	Practical
Tetrahydrocannabinol	Road Side Drug Testing	0.22–10,000	0.22	Practical
D-Amphetamine	Road Side Drug Testing	0.22–1,000	0.22	Practical
Methamphetamine	Road Side Drug Testing	10–8,000	1	Practical

[a] all units are ng/mL unless otherwise specified. [b] units are expressed here as µg/mL.
[c] units are expressed here as U/mL. Table is adapted from: Sensors 2012, 12, 15467-15499.

Table 1: Initial specifications obtained with laboratory-based p-BNC porous bead-based approach: List of developed biomarker assays[1-10], targeted use, and device performance characteristics in the context of real-world clinical testing.

Public private collaboration

Founded in 1961, Porex is the pioneer and global leader in the development and manufacturing of advanced porous materials. The Company is an ISO 9001 certified custom original equipment manufacturer (OEM) of porous polymeric components for filtration, wicking, diffusion, venting, media support and many other applications. Porex is widely recognized for its materials science expertise and proprietary designs which serve over 1,250 customers across more than 60 countries via operations in North America, Europe and Asia.

Utilizing a proprietary sintering process, Porex specializes in forming complex three-dimensional structures and sheet materials using a number of thermoplastic resins. The resulting uniquely bonded structure of Porex components contains desirable characteristics such as depth filtration and robust strength. Due to the strength of the final product, Porex materials prove easy to handle and can be inserted into customer designs using common techniques such as press fitting, heat staking, and ultrasonic welding.

MiniFAB has established a unique, and ISO 13485 certified, development and manufacturing capability dedicated to the manufacture of polymer microfluidic devices and has been providing this service to research institutes and commercial enterprises since 2002. MiniFAB has experience in taking a number of products from concept through to manufacturing stages and currently manufactures in excess of 1 million medical diagnostic devices per annum. Using a structured stage development process MiniFAB works with many groups to transition diagnostic assays onto a disposable platform that enables cost effective, POC testing of patient samples. The development process follows six prescribed steps; detailed product definition and risk analysis (Stage 0), technical risk reduction by demonstrating proposed solutions in proof of principle devices (Stage 1), integration of all cartridge functionality into an concept demonstration prototype (Stage 2), evaluation of manufacturing tolerance on performance and verification of design (Stage 3), implementation of volume manufacturing and validation of design (Stage 4), and scale up of manufacturing (Stage 5). Throughout this process MiniFAB employs design for manufacturing (DFM) to ensure a smooth transition between stages and to eliminate technical risk early in the process.

Conclusions

Few academic medical microdevice concepts have moved all the way to real-world clinical practice progressing through rigorous clinical trials with the inclusion of novel biomarkers. Transitioning microfluidic diagnostic devices from the research to the ISO 13485 regulated commercial environment is a critical process in the product development cycle and requires excellent communication between groups of experts. Whilst application expertise resides with the technology developer or bioscience researcher, the design for manufacturing of disposable polymer cartridges is better handled by a specialist service provider such as MiniFAB. A value added benefit arising from the interaction with such a service provider is the access it provides to an established network of suppliers, as in the case of Porex whose range of materials and expertise in a highly specialised area adds critical function to such devices.

The p-BNC cartridge has successfully transitioned from a prototype device produced in modest volumes within a university laboratory to a device designed for mass manufacturing, enabling an ongoing supply of cartridge consumables to support the development and demonstration of key diagnostic assays on a versatile, low cost platform. These efforts have potential to open up new opportunities for remote multiplexed and multiclass clinical testing through the creation of standardized diagnostic test ensembles that can be quickly customized for new bioscience research and clinical applications.

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