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Pouch-Chip Immunoassays and Nucleic Acid Amplification Tests

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Introduction

Microfluidics technology provides miniaturized fluidic systems for sample processing and analysis, and enables new paradigms in medical diagnostics, especially with regard to in vitro assays of biological specimens performed outside of laboratories. Such point-of-care (POC) diagnostics devices offer low-cost (< $10 per test), rapid (<60 min), easy-to-use tests for infectious diseases in doctors and dentists offices, pharmacies, school infirmaries, and rural clinics, as well as home testing [1].

Progress in microfluidics technology, along with developments in assays, sensors, smartphones, lyophilization and novel detection methods, as well as new rapid prototyping tools (e.g. 3D-printers), are bringing POC tests closer to practical realization and commercial viability. In a common implementation, a palm-sized, plastic, disposable microfluidic substrate called a 'chip' (or cartridge or cassette) hosts a network of conduits, channels and chambers for performing various 'unit operations' such as sample metering and aliquoting, mixing, separations, reactions, and analyte detection [2]. There is as-yet no broad consensus on the best designs, materials, fabrications methods, and modes of operations for microfluidic systems, in contrast to, for example, semiconductor microelectronics where the technology paradigm (silicon, metal-oxide-semiconductor field effect transistor, lithography, digital logic) has been established for many decades. Accordingly, consequently, there is a wide choice of technology options available to implement laboratory assays on a chip. For POC applications in resource-limited settings, there is incentive to reduce or eliminate companion instrumentation and simplify operation suitable for non-professional or minimally-trained users.

Here, we describe microfluidic chips with integrated flexible pouches and membranes that provide fluid reservoirs, fluid actuation (pumping action) and flow control (valving). Such pouch-chips accommodate various levels of sophistication, ranging from manual operation to more automated, autonomous processing, according to the appropriate degree of supporting instrumentation and user intervention desired for a particular application.

In the realm of in vitro medical diagnostics, microfluidic 'lab-on-a-chip' (LOC) devices process clinical specimens such as blood plasma, oral fluid (saliva), and urine for sensitive and specific detection of biomarkers of disease. In POC immunoassays, samples are loaded, minimally processed, and target analyte(s) are immunocaptured or sequestered, washed and immunolabeled for detection. Immunoassays can detect pathogen-associated proteins such as viral and bacterial antigens or host antibodies raised against pathogens. Lateral flow strips represent a particularly elegant and simple implementation of immunoassays, as for example, low-cost home-use tests for pregnancy, drugs-of-abuse, and HIV antibody in sample types ranging from blood, urine, and oral fluid. Lateral flow strips are also used for assaying cardiac markers, pesticide contamination, food and water contamination, and influenza antigens [3,4]. Enhanced sensitivity and specificity, multiplexing (detection of more than one analyte), sample fractionation, e.g. plasma separation from blood, could be gained by microfluidic formats that enable wash steps, reagent mixing, and more optimal interaction with reporters [5].

Molecular diagnostics, more descriptively referred to as nucleic acid amplification tests (NAATs), extract and isolate nucleic acids from clinical specimens such that pathogen-specific nucleic acids sequences can be selectively amplified by enzymatic reactions such as PCR (polymerase chain reaction) to facilitate their detection by optical (fluorescence, luminescence, absorption, color change or turbidity) or electrochemical means. Molecular tests are considerably more sensitive and specific than immunoassays, but at the cost of additional sample processing and accompanying instrumentation. New isothermal amplification methods such as LAMP (Loop-mediated Amplification) use constant-temperature incubation rather than the precise temperature cycling required with PCR, and thus eliminate the need for temperature control instrumentation, allowing considerable simplification of POC NAATs.

Ideally, POC diagnostics chips should be self-contained such that the user merely adds the sample to be tested at the time of use. All reagents, buffers and other liquids are pre-stored on the chip, relieving the user from any manual operations for a convenient "sample-in/answer out" diagnostic device. Enzymes and other reagents are lyophilized and stored in the chip during assembly, and the chip is provided with liquid reservoirs such that buffers and liquids can be delivered during sample processing.

The feasibility of microfluidic-based nucleic acid isolation, enzymatic nucleic acid amplification, real-time or end-point detection, as well as basic processing for immunoassays are well-established [1,6]. Less clear are issues related to optimal implementation of sample introduction and in general, dealing with crude, heterogeneous and variable sample types such as whole blood, and in particular integrating on-chip methods of blood fractionation such as extracting plasma or serum from whole blood.

Pouch chips are an attractive design option for autonomous microfluidic devices, providing a means for fluid storage (reservoirs), fluid actuation (pumping), and flow control (valving) [7-10]. By attaching or laminating flexible polymer sheet materials (e.g. polyester) to a hard plastic (acrylic or polycarbonate) chip, liquid-filled pouches and diaphragm type valves can be formed directly on the chip. Pouches or 'blisters' are hemispherical caps that can be compressed to push air or liquid through a channel. A frangible seal acts as a barrier to contain the liquid contents (50 to 1000 µl) inside the pouch and upon

'activation', the seal is broken providing a fluidic path between the interior of the pouch and the connecting channel. Flexible sheet can also be used to realize diaphragm valves that constrict flow in channels or seal chambers for processing. Chip design, materials, fabrication, and accompanying instrumentation and actuators are presented in detail [8-10].

Pouch chips have been demonstrated for both immunoassays [11-13] and NAATs [6]. Figure 1 shows a pouch chip immunoassay utilizing a conventional lateral flow strip. The microfluidic components of the chip provide means for sample metering and mixing, and a consecutive flow operation where sample loading, washing, and labelling can be affected in separate steps. These features improve the specificity and sensitivity of the assay. This device also employed up-converting phosphors as labels for enhanced detection. Pouch chip NAATs are more complicated.

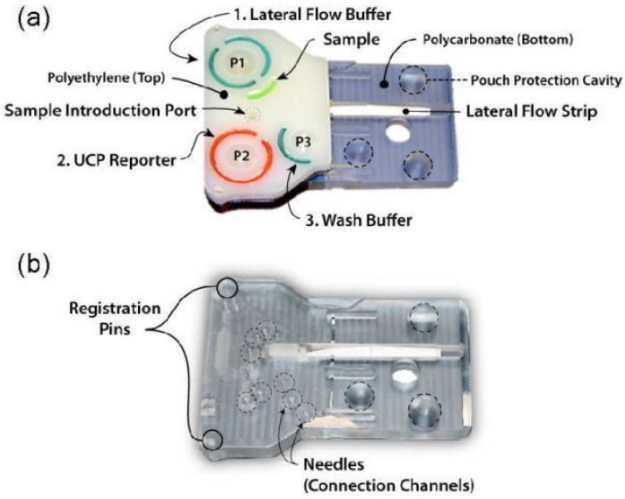

Figure 1: Pouch chip immunoassay. A standard lateral flow strip immunoassay (immunochromatography) comprises a functionalized porous nitrocellulose strip. At one (upstream) end of the strip is a sample adsorption pad containing dried label (antibody against the target analyte conjugated with a reporter particle to enhance visibility). The opposite ('downstream') end of the strip, a wicking pad to induce capillary flow of the liquid added to sample pad. The nitrocellulose is striped with antibody to capture the labelled sample analyte to form a capture zone (test line). Upon addition of sample, the analyte is bound with label and wicks down the strip. Captured, labelled analyte accumulating at the test line serves as a visible test result. Performance of such lateral flow strips can be improved with respect to both sensitivity and specifity by sample metering, better mixing of reagents, separate wash and labelling steps, luminescent reporters. This chip represents an improvement over conventional lateral flow strip immunoassays by adding separate steps for mixing the raw sample (e.g., saliva) with buffer (Pouch 1), performing a wash step (Pouch 3), and labelling the captured analyte in a separate flow step with antibody-conjugated UCP particles. UCP=Up Converting Phosphor, a fluorescent label that avoids background fluorescence effects.

Figure 2 shows a pouch chip that integrates lysis, nucleic acid isolation, PCR, and lateral flow strip detection. Plan views and cross-sections of the chip are shown in Figure 3. Pouch chips can be manually operated, i.e., finger actuation of pouches [12], operated with a non-electrical mechanical actuator [11] or electromechanically with solenoid plungers which depress pouches and diaphragm valves [13].

The pouch chips described here can be compared with some alternative microfluidic approaches where reagents are injected into the chip using off-chip instrumentation, such as programmable syringe pumps [14-17]. These alternatives are overly complex for many POC applications, and can be difficult to manufacture in low-cost and high-volume. Fluid actuation and delivery by external means entails several drawbacks including: 1) leak-tight fluid connections must be made between the chip and the pump(s), complicating use in the field and compromising reliability, 2) a significant dead volume may ensue, wasting reagents and making manipulation of small volumes difficult, and 3) fluidic connections increase the chances of contamination, leading to false positives. These disadvantageous features are avoided with the pouch chips described here.

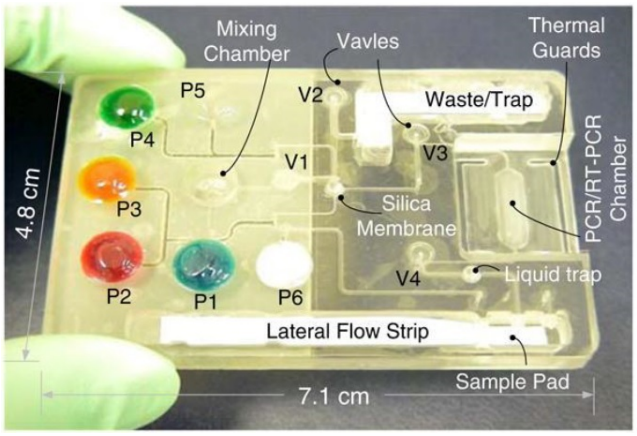

Figure 2: Pouch chip for Nucleic Acid Amplification (PCR) test integrating lysis, nucleic acid isolation by solid-phase extraction, polymerase chain reaction (PCR for DNA targets) or reverse-transcription polymerase chain reaction (RT-PCR for RNA targets), and detection of amplicons on a lateral flow strip mounted on chip. Sample (100 to 200 µl) is added to the chip and mixed with chaotropic salt and detergents which lyses cells and viruses. The lysate is filtered through a permeable silica fiber membrane. The chaotropic salt in the lysate promotes selective adsorption of nucleic acids to the membrane. The membrane is washed with ethanol:water solution to remove proteins and other substances by depressing a pouch reservoir. The nucleic acid is desorbed and eluted from the membrane into a PCR chamber by depressing a pouch. The PCR chamber is sealed by depressing diaphragm valves, and thermal cycled by a programmable-temperature controlled heater plate (not shown) supporting the chip. Post- amplification, a pouch is depressed to empty the contents of the chamber onto the sample loading pad of the lateral flow strip. The primers for PCR amplification are conjugated to capture and label the amplicon on the lateral flow strip test line, which can be visually read. Clear pouches are filled with colored liquids to facilitate visualization of fluid flow.

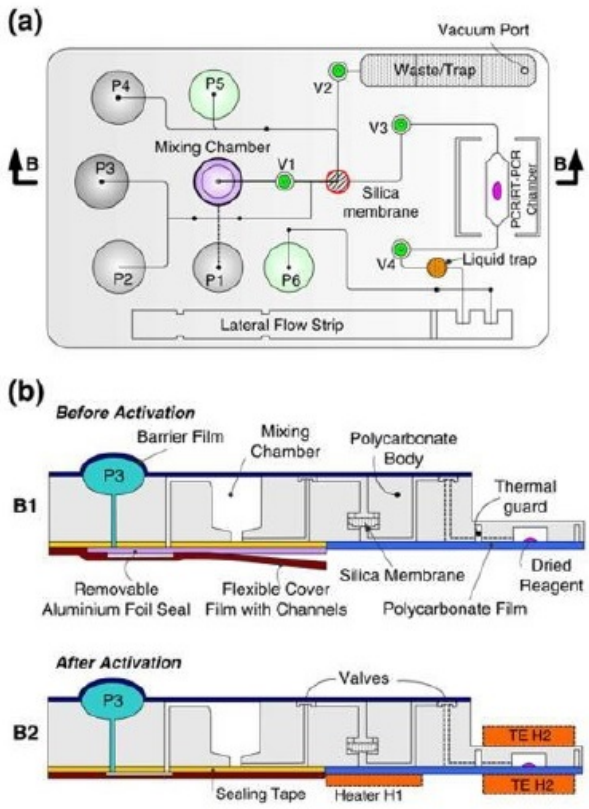

Figure 3: Nucleic Acid Amplification (NAAT) pouch chip integrating lysis, solid-phase extraction for nucleic acid isolation, PCR and lateral flow strip detection. Detailed drawings: (a) top plan view, (b) cross sections [6, 10].

To summarize, microfluidic chips with integrated pouches and flexible membranes provide on-chip fluid reservoirs and one-shot pumping for fluid actuation, as well as diaphragm valves for flow control and chamber sealing. These POC immunoassay and NAAT chips are compact, self-contained, and easy to use, and can be operated manually, with mechanical spring-wound actuators, or with portable instruments equipped with programmed, electromechanical actuators [6,13].

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