EFFICIENT DEVELOPMENT KIT FOR WELL-TO-CHIP CUSTOMIZATION AND DETECTION OF COLORIMETRIC AND FLUORESCENCE BASED MICROFLUIDIC IMMUNOASSAYS

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ABSTRACT

Microfluidics is advantageous for immunoassay processing (e.g., reducing immunoreagent consumption/assay times) [1], but the fact that most clinically relevant bioassays are developed for well-plate technologies poses significant challenges for integration within µTAS platforms. Assays developed for 96-well plates must be downscaled and adapted for microfluidic settings, involving difficulties in volume and surface chemistry optimization in addition to the inability for microfluidic detection using conventional analytical approaches. To address the need for performing replicate immunoassays in microfluidic environments, we report on the development of translational, slide-based microfluidic cartridges integrated with specialized detection technologies (fluorescence and colorimetric) that enable miniaturization/analysis of standard assays.

KEYWORDS: Immunoassay, Supercritical Angle Fluorescence (SAF), Paired Emitter Detector Diode (PEDD)

INTRODUCTION

Traditionally to develop reliable diagnostic assays, copious repetitions of set procedures must be run to ensure correct analysis; well-plates lend themselves nicely for such applications. However, for the development of microfluidic systems, well-plate processing is limited. As opposed to commercially available plates with proprietary protein coating, the materials used for microfluidic prototyping (and in turn mass production) typically require specialized substrates and surface functionalization to link biomolecules that serve as the basis of analyte capture. Furthermore, issues such as the reduction of volumes restrict the use of standard detection equipment to analyze assay processing. This report serves to illustrate systems capable of porting well-plate assays onto microfluidic devices. By using simple rapid prototyping techniques and novel detection methods we have created a kit of tools to enable full development of immunoassays in a microfluidic setting.

CARTRIDGE ASSEMBLY

The cartridges are created by laser machining a laminate of double-sided pressure sensitive adhesive (PSA) and poly(methylmethacrylate) or PMMA sheets (Fig. 1A). In the example shown, an array of microwells is created in the 1.5-mm thick PMMA parts. After production, the carrier layer of the PSA is removed and the top assembly then stuck on glass/polymer slides (Fig. 1B). The slides are modified pre or post assembly with functional biomolecules to create the microarray capture areas. Using these rapid prototyping techniques the end-user can tailor the size/shape/volume of wells in addition to the substrate materials to meet the needs for the various steps of assay development (Fig. 1C). The chips can be made in mass, are ready to use on-demand, and are disposable. Furthermore, the simple cartridge design enables novel detection technologies to be employed during assay optimization.

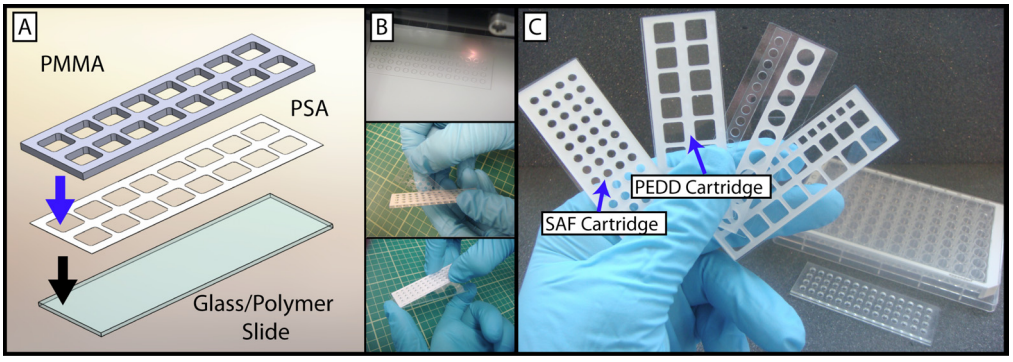

Figure 1: A) Schematic of the assembly. B) PMMA and PSA layers fabricated using laser cutting techniques and bonded to select slides for experimentation. C) Customizable microcartridges for varied analysis of immunoassay miniaturization.

DETECTION TECHNOLOGIES

To address the majority of immunoassays which are based on either fluorescence or colorimetric analysis, two distinct detection technologies were created. For fluorescence-based immunoassays we developed a compact reader system based on a *supercritical angle fluorescence* (SAF). The SAF optical element is unique among fluorescence detection by collecting predominantly emission from within a certain angular range of the critical angle of water/olefin copolymer interfaces [2]. This allows the selective detection of the fluorescence from molecules that are bound to or in close proximity to the solid-liquid interface of the substrate, but excludes detection of emission the bulk solution (Fig. 2).

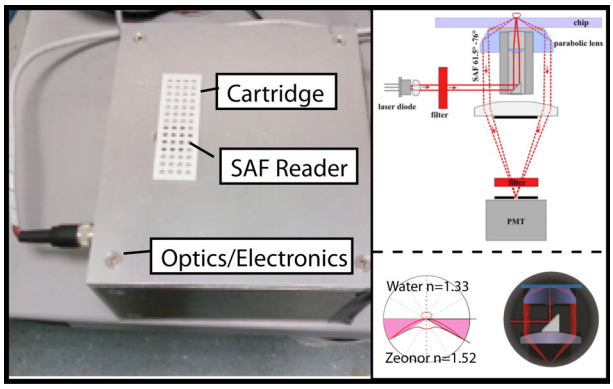

Figure 2: SAF reader and chip. Slides were spotted to create microarrays, cartridges assembled on top followed by running the immunoassay procedure. Slides could be directly analyzed by placing them on the SAF reader.

For colorimetric based-assays a *wireless paired emitter detector diode* device (PEDD) was used. The PEDD system consists of two light emitting diodes (LEDs), placed above and below the sensing area; one LED acting as the light source while the second acting as a detector. In order to make a measurement, the system measures transmittance of light through a sample by analyzing the time taken for the photocurrent generated by the emitter LED to discharge the detector LED [3]. Concentrations of analytes on a chip surface can thus be deduced by examining the digital signal output of the device. The system is highly portable utilizing a lithium battery and able to transmit data wirelessly in real time via Bluetooth (Fig. 3).

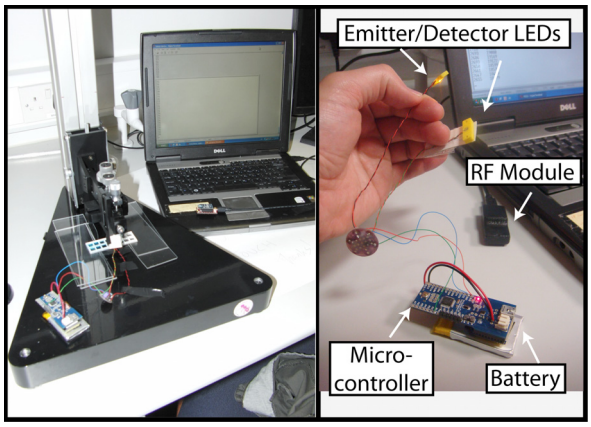

Figure 3: PEDD reader and chip. After the preparation of samples, substrate solutions were loaded into chip. The cartridge was then placed between the PEDD detector and then analyzed for colorimetric evaluation.

EXPERIMENTAL

To validate SAF/PEDD detection using the cartridges, immunoassay experiments were performed versus standard benchtop detection instrumentation. Both fluorescent and colorimetric variants of IgG sandwich assays were created. For fluorescent analysis, APTES (3-aminopropyl triethoxysilane) coated Zeonor slides were pre-printed with goat anti-human IgG antibodies. Then, a highly dense 56 microwell construction was assembled on top. This was followed by adding analyte IgG standards, and Cy5-labelled anti-human IgG with subsequent washes. The cartridge was analyzed by examining the performance of the SAF instrument versus a customary array scanner (Perkin Elmer GX). For the colorimetric assays, a 16-chamber well design was assembled on commercially available Nunc™ microscope slides. After assembly a standard ELISA assay protocol was run in the wells; differing from the fluorescence assay through HRP-labeled secondary antibodies (and using TMB substrates). Colorimetric analysis was monitored using the PEDD detection system and compared to measurements from a commercial microplate reader (Tecan Safire2).

RESULTS AND DISCUSSION

Results showed good correlation between the microfluidic and traditional systems. As seen in Figure 4, the SAF reader measured similar fluorescence intensities as the array scanner (measured over a range from 125 pg/mL to 100 µg/mL). The

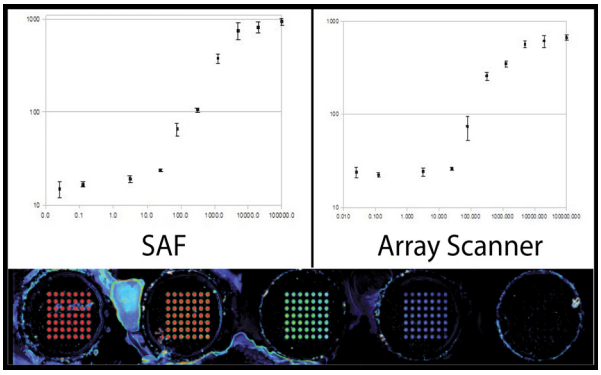

Figure 4: Fluorescence experimental results for the SAF reader and the array scanner. Similar standard curves were visible. Bottom image shows the arrays in the microwell structure.

PEDD system also behaved as the bench top colorimetric instrument, showing comparable measurements in analyte concentration over IgG assay ranges between 0.01 -10 µg/mL (Fig. 5). In this case, a direct inverse relationship was seen due to the fact that the PEDD system measures transmittance while the Tecan machine measures absorbance. In general, this work demonstrated the ability to use the cartridges and the microfluidic detection instruments in immunoassay analysis.

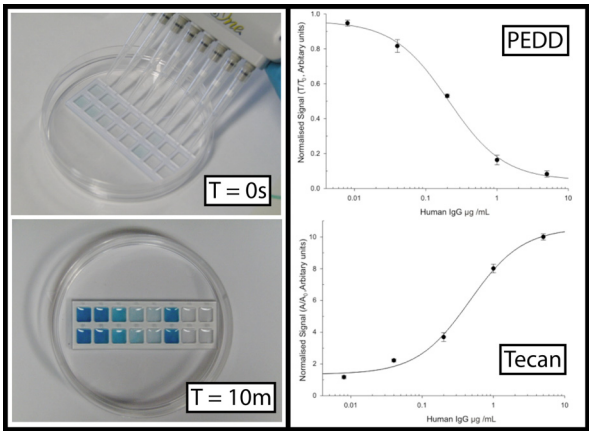

Figure 5: Colorimetric ELISA experiments. Color change due to concentration occurs around 10 mins PEDD showed a direct inverse correlation to the Tecan analyzer (transmittance vs absorbance).

CONCLUSION

The systems reported here allow flexibility in immunoassay miniaturization, being amenable to rapid prototyping and adaptable with commercial reagents/slide components. The cartridges are useful for integrating assays into microfluidic settings; the technology is straightforward to fabricate/use and the assemblies are inexpensive and disposable. Furthermore the use of the cartridges and the SAF/PEDD systems offer new detection methods during the development of integrated microfluidic devices. Potentially, the novel detection strategies could be utilized for reliable diagnostics in point-of-care settings.

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