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RAPID PORTABLE DIAGNOSTIC PLATFORM FOR TOXIN/PATHOGEN EXPOSURE SCENARIOS

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ABSTRACT

A number of emerging pathogens and toxins are significant concerns as possible natural outbreaks or intentional bioterrorism. Timely and specific diagnostics are needed at the point-of-incident or point-of-care to effectively manage health care for outbreak/exposure scenarios. We report on microfluidic technology and systems engineering to realize a point detection medical diagnostic platform to meet the needs for exposure scenarios. The platform enables rapid (10 min), cost-effective and specific detection of up to 64 toxin/pathogen and pre-symptomatic host-response protein biomarkers simultaneously with a drop of blood thus enabling effective countermeasures, including therapeutic intervention, at the earliest stages of intoxication. The platform technology is adaptable for any number of biological threat/host-response panels including routine screening for presymptomatic biomarkers of diseases such as cardiac, septic shock, and cancer as previously demonstrated with clinical saliva samples for detection of MMP-8 a potential marker of active periodontal disease.

INTRODUCTION

As the population, commodities and technical information are ever more fluid across the globe, the chances of a successful intentional or accidental biotoxin attack are increasing. Clinical and public health laboratories have been assigned key roles in the detection and identification of potential assaults using biological threat agents. For biological threat agents, prognosis is poor once the distinguishing clinical signs manifest [1,2], making early diagnosis critical for effective treatment of exposure. Medical devices that rapidly diagnose (or triage) potentially exposed populations are clearly critical for effective countermeasures. A rapid and portable diagnostic method will be useful in many scenarios including direct toxin exposure in populated buildings, transportation hubs, and sporting events where quick triage of a large number of people is vital. We have recently reported on a microfluidic platform designed to quantify biomarkers with a drop of sampled fluid collected non-invasively (saliva, blood, etc) within 10 minutes total assay time. The platform was demonstrated with clinical saliva samples for quantifying levels of MMP-8 a potential presymptomatic biomarker of active periodontal disease [3]. We are currently addressing the needs for rapid, cost-effective, specific, point of care diagnostics of exposure to enable effective countermeasures including therapeutic intervention at the earliest stages of intoxication [4] based in part on earlier systems engineering of portable detection systems [5,6].

The platform relies on microdevice-based electrophoretic separations of protein signatures using fluorescent reporter probes that specifically bind to target analyte (antibodies, aptamers, etc,) or are specifically modified/cleaved by enzymatic activity of target analyte (SNAP 25 substrate for BoNT). The assay format is solution-phase, simplifying assay development, incubation times are short (as compared to solid-phase immunodiagnostic systems such as ELISA), and no wash steps or secondary labeling steps are required. A portable (4"x8"x8"), self-contained device (currently under development, Figure 1) houses the needed hardware (integrated electronics, power supplies, miniaturized optics, fluid handling components, LCD interface, and data acquisition software) to detect multiple reporter probes (4-color), in multiple microfluidic separation channels (8-channels) simultaneously. The total analysis time for a full panel of biomarkers is 10 minutes (for multiplexed individual assays ranging from 3-10 min).

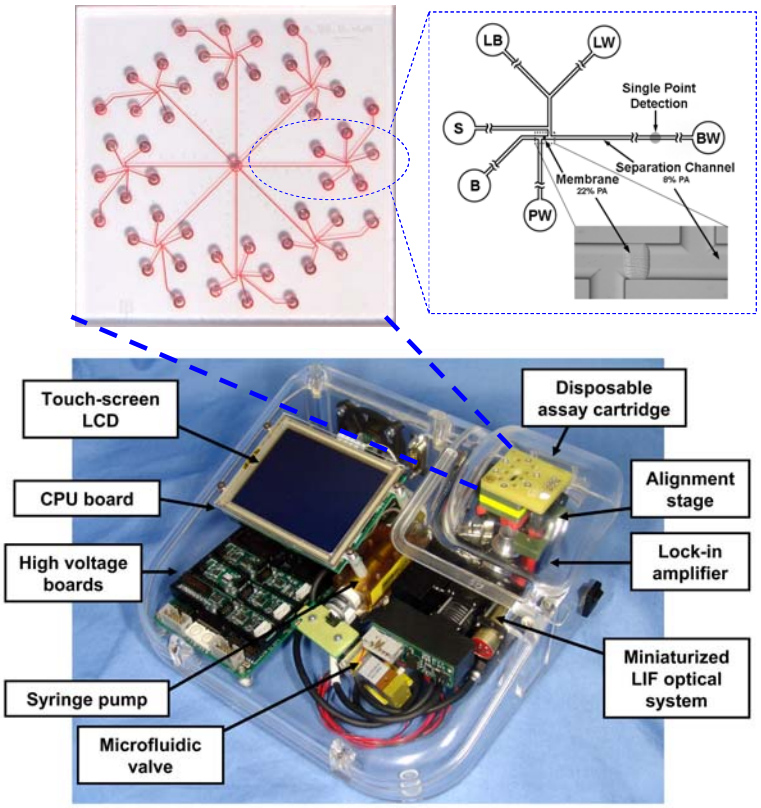

Figure 1. Portable medical diagnostic platform. Self-contained device (below) with key system components labeled. The 1" square microfluidic chip (above) is housed within a disposable cartridge that also houses reagents and electrodes. The multiplexing chip contains 8 separation channels (each separating multiple reporter probes). The inset shows a layout of photopolymerized preconcentration/mixing membrane, sieving gel and point of LIF detection.

The microfluidic chip incorporates multiple photopatterned polyacrylamide gel elements (Figure 1), facilitating sample filtration, target enrichment, on-chip mixing of sample with detection antibody, and electrophoretic separation [3,4,7]. Proteins are driven electrokinetically to nanoporous size-exclusion membranes that trap, preconcentrate, and simultaneously mix sample proteins with reporter probes. By preconcentrating analytes up to 4 orders of magnitude within a 1-5 minute step, detection sensitivity ranges from ~10 fM to nM depending on quality of reagents and desired diagnostic range for each analyte.

EXPERIMENTAL

We have previously described methods for fabricating microfluidic chips and

conducting electrophoretic separation assays [3,4]. Briefly, devices were fabricated with glass using standard photolithography and wet etch techniques. Glass devices with 30 µm isotropic etch resulting in channels 80 µm wide at the top, and 20 µm wide at the bottom were fabricated by Caliper Life Sciences (Mountain View, CA). Microchannel surfaces are treated with 3-(trimethoxysilyl)propyl methacrylate (Aldrich, St. Louis, MO) to provide a point of attachment for polyacrylamide gel. Next, gels are photopolymerized within microchannels by filling channels with a solution of acrylamide monomer, N,N-bisacrylamide crosslinker, and VA-086 photoinitiator in 1X Tris-Glycine buffer (BioRad, Hercules, CA). Three distinct polymers are formed sequentially with different concentrations of monomer. First, a nanoporous polyacrylamide size exclusion membrane (22% T, 6% C), subsequently, a separation gel (6% T, 5% C), and finally, a low-concentration loading gel (3.5% T, 5% C). The region downstream of the preconcentration membrane was left open (no gel). Antibodies are labeled with Alexa Fluor dyes (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. Immunoassays were conducted in 1X Tris Glycine buffer (BioRad, Hercules, CA, USA). Custom high-voltage power supplies electrokinetically drive all manipulations of proteins on-chip. Custom laser-induced fluorescence detection module is designed to scan the 8 separation channels in rotary fashion generating electropherograms at a fixed location in each channel ranging 3-6 mm from the injection point.

RESULTS

A number of assays have been developed for rapid identification of trace levels of toxins (BoNT, ricin, SEB, shiga) and host response markers (IL6, IL2, TNFα, IFNγ) in blood samples. Approaches for sample prep are currently under evaluation and assays are being refined in preparation for animal model studies. Shown in Figure 2 are representative electropherograms for enzyme activity assays and immunoassays.

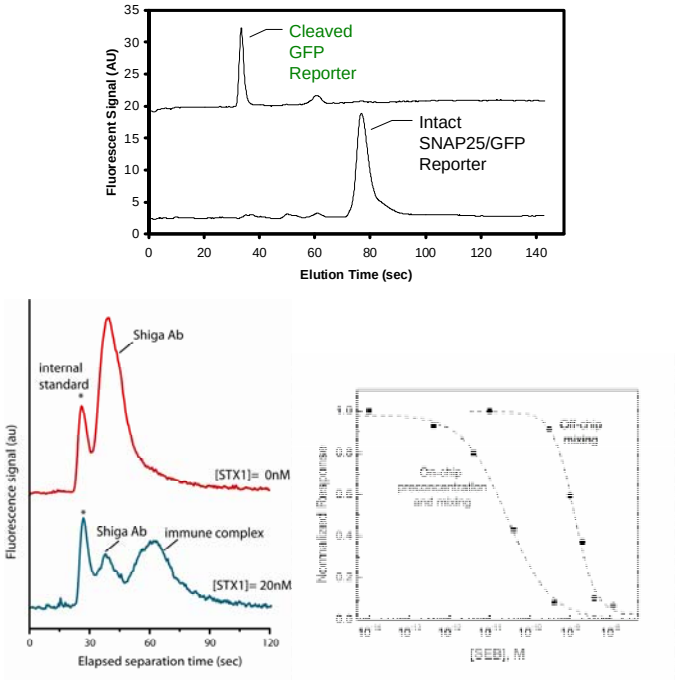

Figure 2. Representative electropherograms for an enzyme activity assay (above, BoNT) and an immunoassay (below, Shiga toxin). BoNT activity is measure by the relative level of cleaved reporter peak vs. intact peak. For immunoassay separations, the unbound reporter antibody peak is reduced in the presence of specific analyte by formation of complex (where peak is increased). The calibration curves for SEB immunoassays demonstrate that sensitivity and detection range are tunable by adjusting preconcentration time.

Also shown in Figure 2 are calibration curves for conditions with and without preconcentration of sample proteins at a size exclusion membrane. The detection limit can be adjusted across several orders of magnitude by simply adjusting the preconcentration time. Preconcentration factors of ~10,000 are achieved within 5 minutes.

We have demonstrated simultaneous electrophoretic separations within all 8-channels of the 1" square multiplexing chip (Figure 2). Up to 49 reservoirs are electronically addressed to drive all assay operations. A maximum of 24 reservoirs are addressed for a given step of the assay with at least 8 of those being grounded. Because assays can be successfully driven in many cases by the same voltages, a single power supply can be shared for up to 8 regions of the chip. The total number of simultaneous assays possible with a combination of 8-channels x 4-color detection x 2 assays per color for a total of = 64 total assays within 10 minutes.

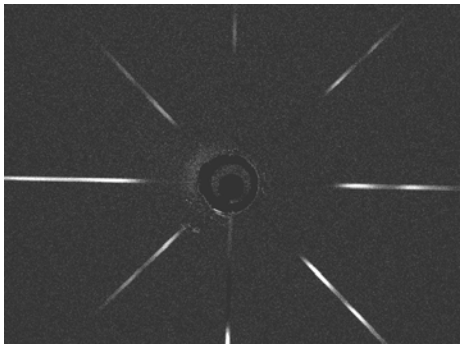

Figure 3. Spatial multiplexing with 8-channel chip. Fluorescence micrograph showing simultaneous separations of analyte within all 8 separation channels. Reporter probes are approaching the central waste reservoir. Note that LIF detection module measures reporter signal at single points within each channel generating an electropherograms used to quantify biomarker levels.

CONCLUSIONS

We have reported on a simple and versatile platform approach to rapid medical diagnostics at point-of-incident/point-of-care. With assays relying on simple gel-shifts of fluorescent reporter, demonstrated options for reporter molecules are versatile ranging from typical antibodies, to aptamers, to enzyme substrates. Demonstrated uses include detection of multiple toxins and host-response markers applicable to both exposure scenarios and screening for disease. The panel approach will greatly aid triage efforts and effective therapeutic intervention by targeting multiple pathogen/toxin signatures and host-response markers simultaneously. As an example, simultaneous enzyme activity assays as well as immunoassays for BoNT provide comprehensive information on presence and toxicity of BoNT including serotype, specific activity and whether both light and heavy chain are present as required for cell entry, thus allowing rapid assessment of whether valuable prophylactics are needed and which type may be most effective.

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