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SpinDx™: A Rapid, Point-of-Care Biodosimetry Platform Using Peripheral Whole Blood Protein and Leukocyte Biomarkers

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Overview

Here we present development of the SpinDx™, a fully-automated, deployable platform for assessing radiation exposure dose based on protein and hematological biomarkers present in peripheral whole blood. Small volume whole blood samples (~20μL from finger puncture) are analyzed with high sensitivity and wide dynamic ranges with a total sample-to-answer time of < 10 minutes. Central to the platform is an embedded disk in which whole blood samples are routed and analyzed within microfluidic channels by centrifugal force. We have developed two innovative assay techniques capable of parallel white blood cell separation and counting, and multiplexed protein quantification via immunoassay. Both assay techniques rely on simple, fundamental principles of sedimentation, thereby providing robust and reproducible diagnostic results. Preliminary studies with a mouse model whole body irradiation study show strong correlation between SpinDx™ results compared with gold standard clinical analysis.

Protein and Hematological Biomarkers of Radiation Exposure

Ongoing research at AFRRI has revealed a patent-pending panel of radio-responsive protein biomarkers present in peripheral whole blood (Ossetrova et al., 2007, Ossetrova and Blakely, 2009, Ossetrova et al., 2010, Blakely et al., 2010). In particular, these studies have shown the statistical power of quantifying multiple biomarkers to estimate dose. For example, combining quantification of the acute phase protein serum amyloid A (SAA) with the ratio of neutrophils to lymphocytes (ANC:ALC) in whole blood provided dose assessment with ~0.5 Gy accuracy across a 1–7 Gy dose exposure range in a BALB/c mice model (Ossetrova et al., 2010). However, traditional assay methods for such biomarkers do not meet the stringent capabilities needed for exposure scenarios. We thus established the following device requirements for a deployable biodosimeter using protein and hematological biomarkers from peripheral whole blood:

1. Combined, multiplexed protein quantification and cell counting capability.
2. Finger puncture (~20-40μL) whole blood sample input
3. Less than 10 minute sample-to-answer time.
4. Fully-automated, requiring minimal user interaction.
5. Portable, hand-carried device.

Sedimentation-Based Clinical Diagnostics

To address the above project requirements, we have developed two novel assay techniques based on fundamental principles of sedimentation within centrifugal microfluidic devices to achieve multiplexed protein immunoassays and white blood cell separation and counting using a single device (Schaff and Sommer, 2011, Schaff et al., 2010). An assay disk is pictured in Figure 1. Microfluidic channels are embedded in polymer disks of similar dimensions as compact disks by either injection molding techniques or by sandwiching pressure-sensitive adhesive between two polymer substrates. Samples are routed through the disk via centrifugal forces as the disk is spun. Capillary action (wicking) and

geometrical design are also used to route the sample from a single inlet port and meter specific sample volumes to each channel.

Both assay techniques are governed by Stokes' Law, which defines the sedimentation rate of a particle in solution as $U_S = \frac{2}{9}\frac{(\rho_p - \rho_f)}{\mu} gR^2$, where ρ_p is the particle density, ρ_f is the fluid density, μ is the fluid viscosity, g is the acceleration rate, and R is the particle radius.

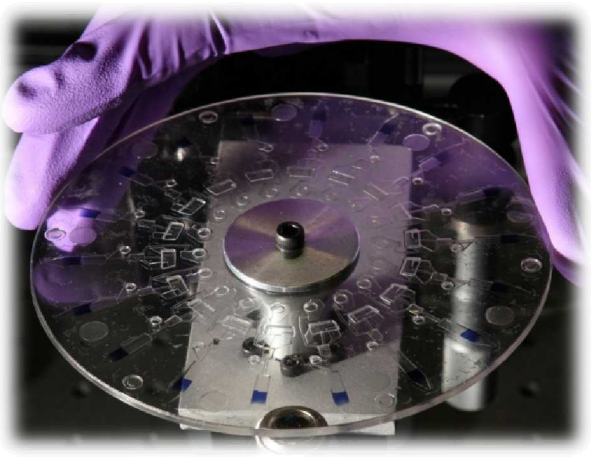

Figure 1. SpinDx™ assay disk.

Centrifugal Leukocyte Counting

Centrifugation is a well-established method for separating and counting white blood cells in the laboratory. To leverage this approach as a diagnostic, we first mix the whole blood with a labeling agent (such as a membrane-permeable DNA dye to fluorescently label each leukocyte in the sample), then layer the sample upon a pre-loaded density gradient within an on-disk channel. Upon spinning the disk, the cells separate into defined bands along the gradient based on their different densities. Following centrifugation, the disk is fluorescently imaged or scanned to quantify the fluorescence of each cell band and calculate the number of each cell type in the sample. Further details regarding this assay can be found in (Schaff et al., 2010).

We have recently applied this technique to the hematological analysis of blood from mice exposed to total body irradiation ranging from 0 – 14 Gy, and sample draws ranging from 4 hr – 4 days post-exposure. On-disk analysis correlates well with data obtained from a veterinary hematology analyzer.

Multiplexed Immunoassays

We have expanded this sedimentation approach into a simple immunoassay approach, as detailed in (Schaff and Sommer, 2011). Briefly, samples are mixed on-disk with a detection suspension consisting of microparticles (i.e., 1μm silica beads) coated with a capture antibody, and a fluorescently labeled detection antibody in solution. Upon a brief incubation, the detection antibody will bind to the microparticle in the presence of the target analyte, forming a sandwich construct. The sample/detection suspension is then layered upon a pre-loaded density medium and centrifuged such that the microparticles sediment out of solution and form a compact pellet at the end of the channel near the periphery of the disk. The remaining sample and detection components (i.e., plasma, blood cells, unbound detection antibody, and other contaminants) remain suspended above the density medium and thus removed from the detection region. This final property results in very low assay sensitivities as the capture substrate is removed from the sample and inherently washed by the density medium, providing high signal-to-noise ratios.

We have developed assays for several radio-responsive protein markers, including IL-6, Flt-3 ligand, SAA, CRP, and GADD45α. Dose-response relationships show limits of detection ranging from 0.01 – 3.00 pg/mL, and dynamic ranges spanning > 6 orders of magnitude in whole blood.

A key advantage of this assay technique lies in its compatibility with nearly any complex sample matrix without requiring additional sample preparation. We have analyzed several clinical samples including whole blood, serum, urine, and saliva with no decrease in assay performance.

Parallel Analysis

Both assays are operable with nearly-identical on-disk channel architecture and operating protocols, thereby facilitating simple parallel and multiplexed analysis on a single disk. Our team is currently

developing methods to pre-load and package disks for storage prior to analysis. Assay operation will require simply loading the disk with the sample at one inlet port, then spinning the disk to conduct all assays.

Platform Integration

In parallel with assay development efforts, we are developing hardware and software instrumentation for automated assay operation, detection, and analysis. Disks are spun with a 48V servo motor capable of spin rates approaching 10,000 RPM. Following assay completion, the disks are analyzed with a miniaturized epifluorescent detector mounted on linear translation stages. The detector consists of LED-based excitation, and emission photons are captured by a photomultiplier tube mounted beneath an embedded dichroic mirror. The system is currently at a testbed level, mounted on a benchtop apparatus.

The benchtop system will be transitioned to a prototype hand-carried, point-of-care platform consisting of miniaturized optics and motion control components. The SpinDx^{TM} prototype measures approximately 8" x 8" x 5", and is operated through a programmable touchscreen interface. The system includes a barcode reader for sample and patient tracking, and a thermal label printer to track assay results. The end product will enable fully-automated assay results from a single finger puncture whole blood sample with less than 10 minute sample-to-answer time.

Conclusion

Several ongoing research efforts continue to explore radio-responsive protein biomarkers as a viable biodosimetry approach. However, existing assay techniques do not meet the needs for rapid, deployable diagnostics in the event of a mass exposure radiation incident. Here we are addressing that need through development of centrifugal microfluidic assays that rely on simple, robust principles of sedimentation. We have demonstrated rapid (<10 minute) sample-to-answer multiplexed protein quantification combined with differential hematological analysis. Our system is easily adaptable to detection of new biomarkers from nearly any complex biological sample, thereby serving as a rapidly-configurable platform to aid in biomarker discovery and verification efforts towards an eventual FDA-approved biodosimetry method and device.

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