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A Handheld Medical Diagnostic Device for Harsh Environments

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Disclaimer

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Proposal Title: A Handheld Medical Diagnostic Device for Harsh Environments
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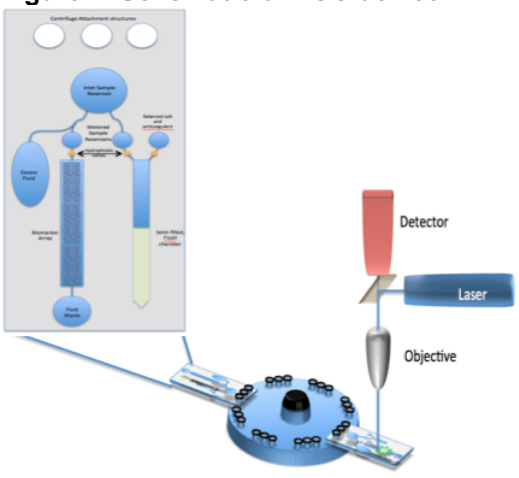
Abstract

Medical diagnostic capabilities for extreme environments, are currently limited in nature to single marker type assays. We propose to develop a handheld device for medical diagnostics in extreme environments that provide sensitive and specific detection of biomarkers with an emphasis initially on radiation exposure. Such a device will have multiple uses, and will be easily adaptable to many applications. For example, first responders, the battlefield, in low resource settings in developing countries facing emerging disease outbreaks, and during long-duration space flights. To this end, we will develop highly adaptable microfluidic devices (cards) for profiling different biomarkers with an emphasis on those related to radiation exposure. We will leverage the previous centrifugal separator work from LLNL and Sandia National Laboratory. The radiation exposure biomarker work at LLNL will provide a biological platform for demonstrating the utility of the device. We will develop a fluidic system using a pump-less mechanical method (centrifugal) to move, control, order and layer fluids in the platform. This decreases complexity, while enabling simultaneous cell separation and plasma generation for two uses: the first is differential blood cell counts and the second is biomarker assay panels. This work will be accomplished in three Aims: **Aim 1)** Test multiplex microfluidic cards and integrated reader. **Aim 2)** Demonstrate system capabilities to take and process blood samples for multi-endpoint analysis. **Aim 3)** Test multiplex detection based on measuring differential blood cell counts, while measuring plasma protein biomarkers. The proposed work addresses the mission research challenge of chemical and biological countermeasure in that our work aims to provide new research capabilities to respond to man-made or natural outbreaks of pandemic diseases in particular in low resource and triage environments.

Figure 1. Schematic of POC device.



Background and Research Objectives

Point-of-care (POC) testing is paramount to proving fast and reliable results for diagnostic and patient treatment in non-hospital, Echelon II locations. Integrating rapid analysis times with high sensitivity and specificity is the cornerstone of microfluidic lab-on-a-chip technologies. This area of research and development enables the miniaturization and integration of central laboratory functions onto a small, compact and portable microfluidic system. *We will develop a compact microfluidic device to separate and count blood cells from small (finger prick) samples, while measuring selected protein biomarkers in extreme environments. The proposed miniaturized detection device shown in Figure 1., would be a key component for future NASA missions beyond*

low earth orbit. The envisioned system is a cartridge-based Lab-on-a-Chip platform capable of sampling biological fluid, preparing and handling the fluid for detection, and measuring fluorescence and optical results. The system will have an integrated dual detection pathway (biomarker detection and blood differential measurements). Ultimately, such a device will be multi-omic capable for detecting cells, nucleic acids and proteins in a single sample.

GOAL: *Deliver a workable prototype triage device that is inexpensive, rapid, easy-to-use and capable of blood cell differential counts and protein profiling for characterizing biological responses of radiation exposure.*

Task 1. Engineer an integrated centrifugation-based microfluidic card and reader system: The design of the microfluidic cartridge system consists of two main components: The fluidic cartridge and the detection system. The cartridge will include 1) fluid sampling and 2) fluidic handling and preparation. The detection housing will include 1) the centrifugal actuator and 2) the optical readout. We will leverage passive flow assays developed at LLNL and the centrifugal force SpinDx technology from Sandia National Laboratory in design of our fluidic cartridge. Using a combination of virtual hydrophobic valving, variable speed centrifugation, strategic venting and optimal radial layout of the chambers and fluidic interconnects, we will control the main fluidic flow, washes and auxiliary fluid volumes, and sample mixing from sample introduction through optical detection readout without the need of pumps. Additive, subtractive and hot embossing are the main identified fabrication methodologies for producing the fluidic cartridge. Each have advantages and disadvantages; however, as part of AIM 1, we will run a systems analysis to determine best path forward for the project and stakeholders.

Task 2. Develop blood handling and processing in a single device: Our fluidic flow system is controlled by centrifugal forces. By manipulating the burst force of the covalently bonded virtual hydrophobic valves (e.g. material properties, valve length along channel and radial location from spin center) we will control when and where fluids flow/mix. Utilizing purchased and/or whole blood obtained from healthy individuals (under an LLNL IRB), Ficoll (or other gradient matrix), wash solutions, and a bench-top optical capture system, we demonstrate controlled fluid flow, cell separation and plasma generation on the cartridge. By incorporating flexibility into the cartridge design we can easily iterate chamber location, valve length and material, and applied force to optimize the cartridge performance.

Task 3. Demonstrate differential blood cell counts while measuring plasma-based protein biomarkers: Red blood cell (RBC), white blood cell (WBC) and plasma separation of whole blood will be verified via optical imaging. *In situ* fluorescent tagging of WBC's along with a fluorescence chip reader, we will measure the ability to separate cell types. Modifying/changing the separation gradient materials and centrifugal spin speeds, we will optimize this cell separation. We will leverage identified health, cancer and radiation exposure biomarkers (available from Coleman lab at LLNL) to create a highly specific and sensitive biomarker array panel. These include panels of biomarkers that are known to be involved in radiation through pathways for cancer, DNA repair signaling and inflammation. Whole blood will be irradiated ex vivo or exposed in vivo (radiotherapy patients) to demonstrate

fluorescently labeled detection in the array panel. Initial experiments will utilize a COTS fluorescence chip reader for detection. Bench-top antibody/plasma experiments will serve our standard and a method to quantitatively verify our centrifugal system in terms of detection limits as well as specificity. Multiple mouse, human cell line and tissue samples could be utilized to further demonstrate the utility of the device using spiked-in controls compared to anticipated ionizing radiation responses.

Scientific Approach and Accomplishments

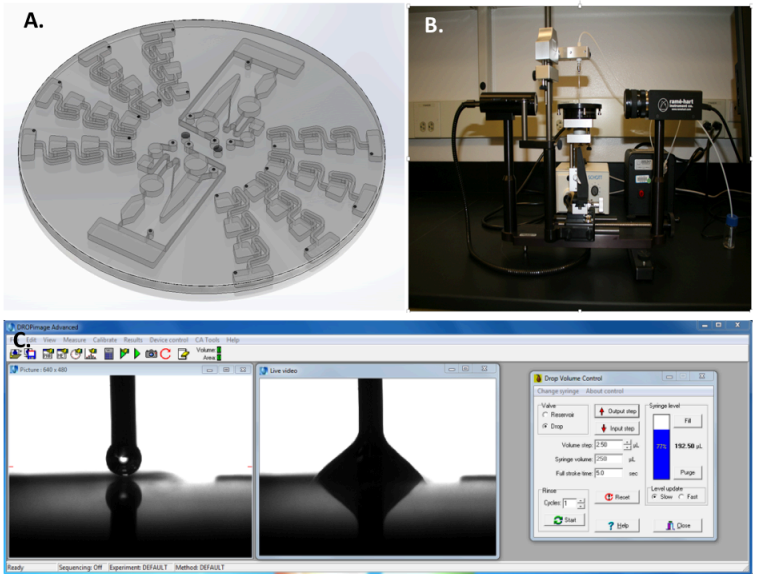


Figure 2. A. Spinning disc design for testing hydrophobic and siphoning valve systems integrated with 2 microfluidic designs for demonstrating biomarker detection and blood cell counting. B. Bench top sample applicator and centrifuge system. The spinning disc centrifuge contains a speed control and camera system for measuring the speed of the disc as well as sample application. C. Sample applicator is demonstration. Microfluidics have been designed to directly deliver sample to the disc surface of the disc and ca be directly images with our bench top system.

Our approach is to engineer a workable prototype utilizing known target and developing biomarkers related to radiation exposure previously identified by our work at LLNL. The new system will be highly adaptable for both performing blood cell counts and profiling protein analytes important for biological-detection of radiation exposure as well as general health. In FY18 we developed an integrated centrifugation-based microfluidic card utilizing the hydrophobic and siphoning valving utilizing a variable speed centrifugation system. The spinning disc has undergone modification and modeling to demonstrate the new liquid handling system (Figure 2.A). We have also built a variable centrifuge system (Figure 2.B) to accurately control the speed of the disc, which affects liquid movement. We have also developed a liquid sampling process to deliver up to 100 uL of liquid (Figure 2.C). This data was presented at the "Human Research Program Investigators Workshop" to the director of the NASA Translational Institute (January 2018) and was used as part of an invited presentation on "Biomarkers for use in early and late biodosimetry" at the EPR Biodose meeting in Munich, Germany.

In FY19 we refined the integrated centrifugation-based microfluidic card works by moving from primarily hydrophobic valving to focus on developing a sophisticated liquid siphoning system to move liquid material. In conjunction with a Capstone engineering project at University of Arizona the entire housing system with a variable speed centrifugation centrifuge and image workstation were designed and built. The schematic for the spinning and

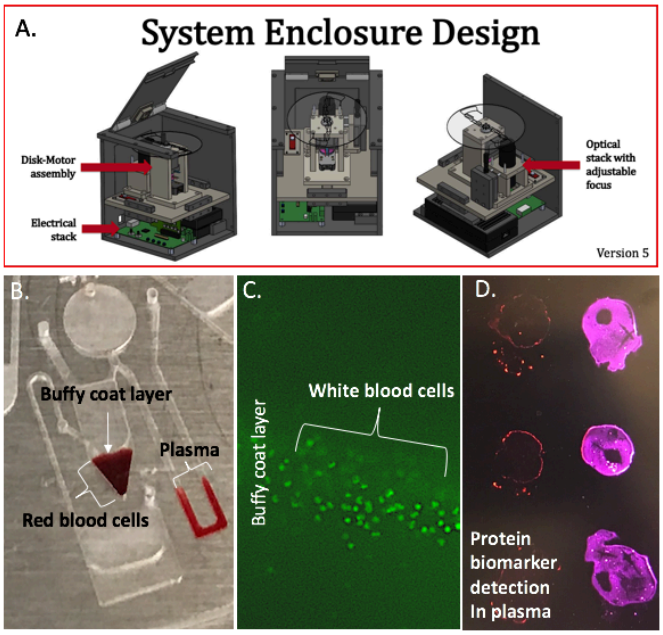


Figure 3. Results from FY19 research. **A.** Schematic of the enclosure that was built at University of Arizona. **B.** Demonstration of microfluidic separation of whole blood in to 3 components (Red blood cells, Buffy coat:white blood cells and plasma). **C.** Demonstration of visualization of white blood cells isolated within the buffy coat using our spinning disc microfluidic technology. **D.** Comparison of background (column 1) and antibody detection (column 2) within the spinning disc microfluidics technology.

imaging box is shown in Figure 3.A. The integrated centrifugation-based microfluidic card was further tested utilizing up to 100 uL of whole animal or human blood for separating it in to three components. First the, buffy coat is separated from the red blood cells, while the plasma and then removed from the top of the buffy coat. The complete separation is shown in Figure 3.B after the whole blood has separated through variable speed centrifugation. Assessments of biomarker sensitivity and specificity were then initiated for white blood cell counts and protein biomarker analysis. Prototypes and data were presented at national and international meetings.

We were also able to initiate genomic and proteomic data analysis on both cells and human-derived samples in collaboration with UC Davis, Travis Airforce Base and NASA. This data will form the basis of future funding and manuscripts regarding radiation exposure and biomarker discovery. Although work was proposed for FY20 to validate the biological markers and fully integrate sample handling and image analysis, the project was administratively stopped at 20 months.

Impact on Mission

The results of this project provide a test bed for producing and characterizing a host of novel proteins for analyzing their molecular and cellular functions. This project showed we were capable of working at the interface of biology, engineering, and the physical sciences to address biosecurity and human health by providing unique diagnostic systems for use variable environments. This project strongly complements ongoing measurement science (biophotonics) and threat biology.

The device and microfluidic cards in its current configuration will be of use for developing future funding from external agency programs similar to past U.S. Department of Health and Human Services, National Institutes of Health (NIH) opportunities (RFA-AI-18-045 Radiation Biodosimetry Assays and Devices) and the Biomedical Advanced Research and Development Authority (BARDA) Biodosimetry program.

Conclusion

There are a number of technical challenges along with the normal institutional challenges and resource limitations that affected the success of the proposed work. The major challenge remains of integrating the microfluidic system with imaging for testing biologically relevant biomarkers. Biological assays would have been refined and further developed in year 3 had the project not been stopped at 20 months.

Publications and Presentations

Invited talk - HemholtzZentrum, Munich, GE (2019): Radiation meets Oncology- Dual use of biodosimetry assays in human cancer treatment and outcome: Characterizing neuroblastoma in patients treated with ^{131}I-mIBG.

Invited talk - CONRAD, Munich, GE (2019): Biodosimetry of internalized irradiation exposures using transcriptional analysis from relapsed and refractory neuroblastoma patients from a NANT11-01 study treated with 131I-MIBG..

Invited talk - BioDose 2018: 22nd Nuclear Medical Defense Conference. Munich, Germany. (2018) "Biomarkers for early and late biodosimetry using lymphocytes from relapsed and refractory neuroblastoma patients treated with ^{131}I-mIBG".

Invited talk - Biomarkers: From Bench to Clinic. University of Arizona. Phoenix, AZ (2018) "Developing biomarkers associated with internalized isotope treatment for refractory and relapsed neuroblastoma".

Master degree Thesis - Sandra Bircher, M.S. awarded October 2018. Development of biologically inspired nanolipoprotein particles loaded with curcumin as tools for radiation protection and mitigation. School of Medicine at the Technical University of Munich.

Master degree Thesis - Tim Setzkorn, M.S. awarded October 2018. Characterizing microRNA expression associated with ^{131}I-mIBG therapy for children with advanced neuroblastoma. School of Medicine at the Technical University of Munich.

Poster presentation – MHSRS, Orlando Florida (2019) Designing and building ruggedized microfluidic cards for point of care applications. Matthew A. Coleman, David Loftus, Michael Triplett, Erik Mukerjee, Jing Li, Tore Straume, Jianing Yang, Alan Nordquist, Frederic Zenhausern, and Matthias Frank.

Poster presentation – MHSRS, Orlando Florida (2019) Development of a point of care technology for military medical applications in austere environments. David J. Loftus, Jing Li, Tore Straume, Matt Coleman, Matthias Frank, Erik Mukerjee, M. Austin Johnson, Ian Stewart.

Poster presentation – NASA Human Research Program, Galveston, Texas (2018) Effect of space flight on astronuats plasma-derived exosomal miRNA expression: implications for biomarker development. R. Kishore, A. Hakobya M. Vangala, V.N.S. Garikipati, A. Khachatryan, L. Nersisyan, P. Mills, M.A. Zuriaga, Matthew A. Coleman, Angela C. Evans, K. Walsh, A. Arakelyan and D. A. Goukassian.

Poster presentation – NASA Human Research Program, Galveston, Texas (2018) Novel models to monitor in vivo effects of SPE/GCR radiation on hematopoetic and GI systems. B. Kuhlman, S.J. Walker, C. Langefeld, T. Pardee, J. Yang, J. Lacombe, M. Saxena. M.A. Coleman, F. Zenhausern, M.G. Almeida-Porada, P.F. Wilson, and C.D. Porada.

Poster presentation – Radiation Research Society, Chicago, Illinois (2018) Early and late response to irradiation exposure of miRNA after exposure to 131I-mIBG treatment. Tim Setzkorn, Haley R. Segelke, Paul Wilson, Andrew Vaughan, Clifford Tepper, Stephenie Liu, Ryan Davis, Katherine K. Matthay, M. Meaghan Granger, Araz Marachelian, Daphne A. Haas-Kogan, Steven G. DuBois and Matthew Coleman.

Poster presentation – Radiation Research Society, Chicago, Illinois (2018) Biologically inspired nanoparticles for radiation protection and mitigation. Sandra Bicher, Wei He, Angela C. Evans, Megan L. Shelby, Paul F. Wilson, Bradford M. Kuhlman, Christopher D. Porada, Graça Almeida-Porada, Thomas E. Schmid and Matthew A. Coleman.

Poster presentation – Radiation Research Society, Chicago, Illinois (2018) Microarray analysis in response to 131I-mIBG treatment using lymphocytes from relapsed and refractory neuroblastoma patients: Identifying biomarker response to irradiation exposure. Angela C. Evans, Haley R. Segelke, Paul Wilson, Andrew Vaughan, Clifford Tepper, Stephenie Liu, Ryan Davis, Katherine K. Matthay, M. Meaghan Granger, Araz Marachelian, Daphne A. Haas-Kogan, Steven G. DuBois and Matthew Coleman.

Notes to the Editors

Nothing to add.