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TITLE: Characterizing Concussion Using Brain Derived Exosomes

PRINCIPAL INVESTIGATOR: David Issadore

CONTRACTING ORGANIZATION: University of Pennsylvania, Philadelphia, PA

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14. ABSTRACT

Traumatic brain injury (TBI) currently afflicts 357,000 enlisted military men and women in the US Armed Services. For the most common form of TBI, Mild Traumatic Brain Injury (mTBI) most patients recover within a year following the incident, but 10-20% of mild cases result in a long-term disability including seizures and emotional and behavioral issues. Although much has been learned about molecular changes in the brain following injury, access to these biomarkers following mTBI is lacking. The accurate diagnosis and precise individual clinical management of traumatic brain injury (TBI) is limited by the lack of accessible molecular biomarkers that are informative regarding the unique mixture of injury mechanisms in each TBI patient.

15. SUBJECT TERMS

Traumatic brain injury, diagnosis

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1. INTRODUCTION:

Traumatic brain injury (TBI) currently afflicts 357,000 enlisted military men and women in the US Armed Services. For the most common form of TBI, Mild Traumatic Brain Injury (mTBI) most patients recover within a year following the incident, but 10-20% of mild cases result in a long-term disability including seizures and emotional and behavioral issues. Although much has been learned about molecular changes in the brain following injury, access to these biomarkers following mTBI is lacking. The accurate diagnosis and precise individual clinical management of traumatic brain injury (TBI) is limited by the lack of accessible molecular biomarkers that are informative regarding the unique mixture of injury mechanisms in each TBI patient.

We hypothesize that we can address this challenge by developing a microchip-based diagnostic to characterize TBI recovery and history using the RNA cargo found in brain-derived extracellular vesicles (EVs). Unlike prior work that has mainly focused on single biomarkers, our approach measures a panel of circulating EV miRNA markers processed with machine learning algorithms to more comprehensively capture the state of the injured and recovering brain. We piloted this approach and successfully classified the severity, time elapsed since initial injury, and history of multiple injuries of TBI in an animal model and with clinical samples. Our proposed chip combines two technologies, developed in my lab, to create an ultrasensitive, automated exosome diagnostic: **1.** Magnetic nanopore isolation of EV subpopulations from the injured and recovering brain, and **2**. Time-domain encoded optofluidics for rapid highly multiplexed digital droplet exosomal RNA detection. Our approach can measure the state of injury and recovery in TBI in a minimally invasive fashion, opening new opportunities to improve molecular diagnosis, prognosis, and precision medicine for TBI injury.

2. **KEYWORDS:**

Mild Traumatic Brain Injury, Diagnostics, Exosomes, Extracellular Vesicles

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

Traumatic brain injury (TBI) currently afflicts 357,000 enlisted military men and women in the US Armed Services. For the most common form of TBI, Mild Traumatic Brain Injury (mTBI) most patients recover within a year following the incident, but 10-20% of mild cases result in a long-term disability including seizures and emotional and behavioral issues. Although much has been learned about molecular changes in the brain following injury, access to these biomarkers following mTBI is lacking. The accurate diagnosis and precise individual clinical management of traumatic brain injury (TBI) is limited by the lack of accessible molecular biomarkers that are informative regarding the unique mixture of injury mechanisms in each TBI patient.

Phase 1

Major Task 1: Next Generation Technology Development

Subtask 1: Finite element design optimization of next generation TENPO.

Intended completion date 6/1/2020. Status: 100% complete.

Subtask 2: Next generation TENPO characterization.

Intended completion date 6/1/2020. Status: 100% complete.

Subtask 3: Develop, test modular magnetic nanoparticle labeling for capturing specific subsets of extracellular vesicles (EVs).

Intended completion date 6/1/2020. Status: 100% complete.

Subtask 4: Validation of EV isolation.

Intended completion date 6/1/2020. Status: 100% complete.

Subtask 5: Isolation of RNA cargo from EVs.

Intended completion date 6/1/2020. Status: 100% complete.

Subtask 6: Develop a droplet detection technology to measure at least three colors allowing ratiometric, calibration free use and expanded multiplexing.

Intended completion date 6/1/2020. Status: 100% complete.

Milestone: Next generation TENPO will be benchmarked for throughput (100 mL/hr), biomarker-selectivity, background insensitivity, and limit of detection (1000 EVs / mL in plasma).

Intended completion date 6/1/2020. Status: 100% complete.

Milestone achieved: Droplet detection platform will be benchmarked for throughput (106 droplets / sec), accuracy AUC > 0.995, and for number of colors (n > 3).

Intended completion date 6/1/2020. Status: 100% complete.

Major Task 2: In Vitro Biomarker Selection

Subtask 1: To screen for surface marker candidates we will use multiple cell-culture based stretch models of injury including models using nearly pure cultures of neurons, astrocytes, and blood-brain-barrier. We will identify identify surface markers unique to each cell type, and RNA markers with high differential expression between injured and control (sham) state.

Intended completion date 6/1/2020. Status: 100% complete.

Milestone Achieved: A set of EV surface markers to isolate subpopulations to profile the injured and recovering brain.

Intended completion date 6/1/2020. Status: 100% complete.

Major Task 3: Pilot Clinical / Porcine Evaluation

Subtask 1: We will isolate multiple extracellular vesicle (EV) subpopulations from injured patients and healthy controls using the TENPO from *N* = 20 injured subjects and *N* = 20 controls.

Intended completion date 6/1/2020. Status: 100% complete.

Subtask 2: We will sequence EV isolated from *N* = 40 banked serum samples from a porcine injury model

Intended completion date 6/1/2020. Status: 80% complete. This work was delayed by COVID-19 shutdown. Samples have been processed and submitted to the sequencing core at University Pennsylvania, and we are currently awaiting results.

Milestones Achieved: We will have sequencing data of the µRNA isolated, from each EV subpopulation, for every patient.

Intended completion date 6/1/2020. Status: 80% complete. This work was delayed by COVID-19 shutdown. Samples have been processed and submitted to the sequencing core at University Pennsylvania, and we are currently awaiting results.

Milestones Achieved: We will have comparisons of this sequencing data to known biological models of injury/recovery.

Intended completion date 6/1/2020. Status: 0% complete. This work was delayed by COVID-19 shutdown. Samples have been processed and submitted to the sequencing core at University Pennsylvania, and we are currently awaiting results. Analysis will be carried out immediately when data becomes available.

Milestones Achieved: Data accumulated from each subject will be annotated with the sequencing data and analyzed.

Intended completion date 6/1/2020. Status: 0% complete. This work was delayed by COVID-19 shutdown. Samples have been processed and submitted to the sequencing core at University Pennsylvania, and we are currently awaiting results. Analysis will be carried out immediately when data becomes available.

Milestones Achieved: We will have sequencing data, from each EV sub-population, isolated from *N* = 40 banked serum samples from a porcine injury model.

Intended completion date 6/1/2020. Status: 80% complete. This work was delayed by COVID-19 shutdown. Samples have been processed and submitted to the sequencing core at University Pennsylvania, and we are currently awaiting results. Analysis will be carried out immediately when data becomes available.

Milestones Achieved: We will have compared this porcine model to our clinical data, validating it it for further use in our study.

Intended completion date 6/1/2020. Status: 0% complete. This work was delayed by COVID-19 shutdown. Samples have been processed and submitted to the sequencing core at University Pennsylvania, and we are currently awaiting results. Analysis will be carried out immediately when data becomes available.

Milestone Achieved: HRPO/ACURO Approval

Intended completion date 6/1/2020. Status: 100% complete.

Milestone Achieved: Meeting with the FDA for guidance

Intended completion date 6/1/2020. Status: 80% complete. We have reached out to the FDA but due to COVID-related delays, have not yet had our meeting. We intend to accomplish this goal this Fall.

What was accomplished under these goals?

Major Task 1: Next Generation Technology Development

Subtask 1: Finite element design optimization of next generation TENPO.

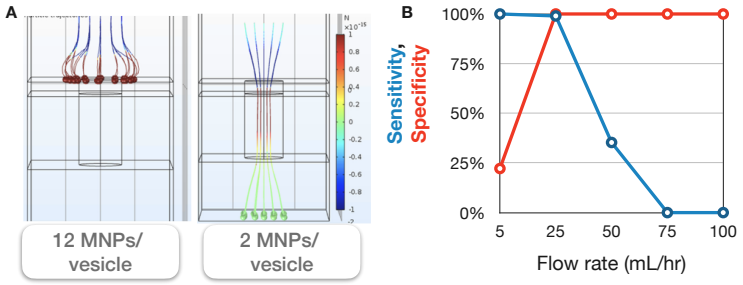

Fig. 1 Finite element simulation of optimization of next generation TENPO. a. An example image of particle tracking of model EVs in TENPO, demonstrating specific capture of 100 nm EVs labelled with 12 MNPs/EV versus non-specifically labelled EVs with 2 MNPs/EV. **b**. By scanning the flow rate, we demonstrate that sensitivity, i.e. the rate of targeted EVs captured, can be traded-off with specificity, the rate of non-targeted EVs properly discarded.

In this sub-aim we used a finite element model of our track etched magnetic nanoparticle device to optimize flow conditions and device geometry. Building on easier work where we had performed a magneto static simulation of our device, we have now incorporated the capability to particle-track individual vesicles traversing our device, using a multi-physics model incorporates magnetostatics and fluid dynamics.(**Fig. 1 a**) This model has allowed us to design out device to choose flow conditions to capture targeted extracellular vesicles without capturing background vesicles with non-specifically bound nanoparticles.(**Fig. 1b**) This work will be included in an upcoming publication.

Subtask 2: Next generation TENPO characterization.

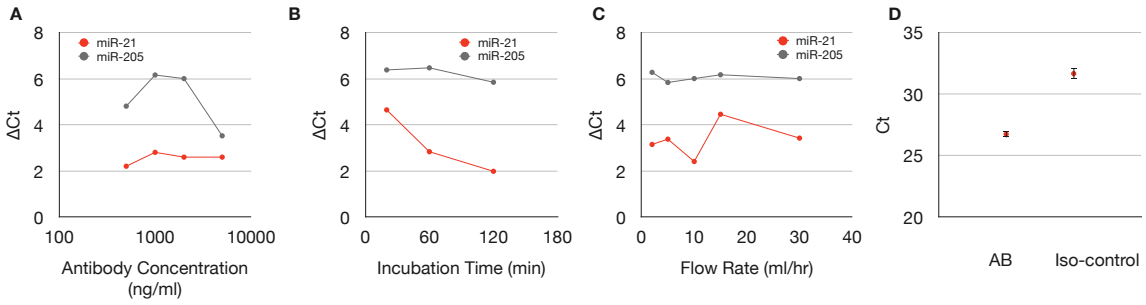

Fig. 2. Next Generation TB characterization. a. Titration of antibody concentration versus difference in PCR cycle number ΔCt, between healthy plasma and healthy plasma spiked with cell culture derived EVs. Plasma and spiked EV concentrations were held constant as measured using Nanoparticle Tracking Analysis. **b.** Using the same model system, incubation time was titrated, as well as flow rate through TENPO (**c**) to identify optimal operating conditions. d. Using these optimized conditions, the recovered RNA was compared to that using a control isotope antibody, to demonstrate significant ($P<0.01$) capture of vesicles versus capture due to non-specific binding.

Building on our computer modeling, we experimentally characterized and optimized the performance of TENPO for surface marker specific EV capture. To this end, we optimized antibody concentration times (**Fig. 2a**), incubation times (**Fig. 2b**), and device flow rate (**Fig. 2c**), using a model system consisting of cell culture derived EVs spiked into healthy plasma in known quantities determined using Nanoparticle Tracking Analysis.

Subtask 3: Develop, test modular magnetic nanoparticle labeling for capturing specific subsets of extracellular vesicles (EVs).

To maximize nanoparticle loading and minimize antibody usage, we are applying an efficient two-step bio-orthogonal magnetic labeling, to enables the use of generic nanoparticles, the efficient use of affinity ligands, and amplified magnetic labeling. We are applying this strategy by labeling biomarkers with affinity ligands modified with trans-cyclooctene (TCO) and then MNPs modified with 1,2,4,5-tetrazine (Tz). We use super-paramagnetic (d < 15 nm) cross-linked iron oxide nanoparticle (CLIO). Cross-linked iron oxide (CLIO) nanoparticles will be used because of their stability, biocompatibility, and lack of non-specific binding.[43] CLIO nanoparticles contain superparamagnetic iron oxide core (10 nm iron oxide) composed of ferrimagnetic magnetite (Fe_3O_4) and maghemite (γFe_2O_3). The metallic core is coated with dextran and cross-linked and functionalized with primary amine. We will explore targeting CD63, CD81, and CD 9 (pan exosone), or a combination thereof or cancer epitopes, using commercial antibodies, each modified with TCO. The labeling efficiency is characterized using NMR T2 relaxometry (Bruker), and we have demonstrated significantly enhanced labelling (P<<0.01) using this method compared to direct labelling.

Subtask 4: Validation of EV isolation.

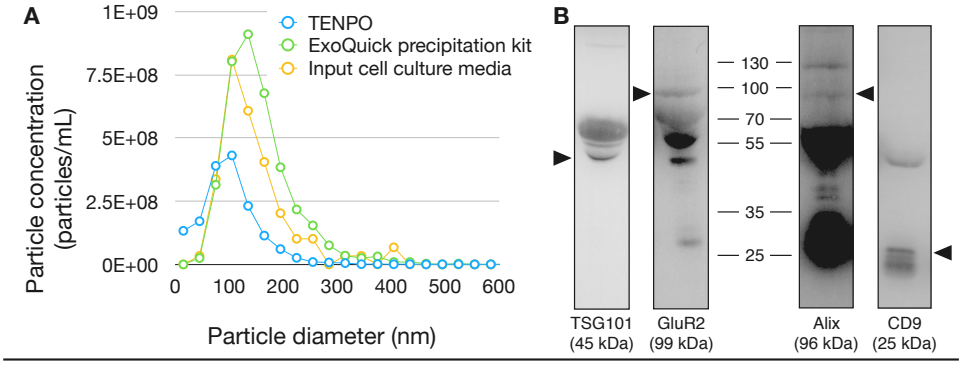

Figure 3. Validation and characterization of EV isolation a. Nanoparticle Tracking Analysis (NTA) data comparing the eluted isolate from TENPO (using CD9, CD61, CD83 pan-exosome cocktail), a commercial exosome isolation kit, and input cell culture media, demonstrating an isolate with sizes consistent with exosomes. **b.** Western blot analysis for the presence of exosomal markers (TSG101, Alix, CD9) and GluR2, a surface protein that we used to capture specific types of vesicles.

To characterize the vesicles captured by TENPO, EVs are isolated from cell culture media using TENPO and pan-exosome surface markers and then characterized using Nanoparticle Tracking Analysis (NTA) and Western blot. The isolate had a size consistent with exosomes, and compared favorably with the isolate from a commercial exosome isolation kit (ExoQuick), and the cell culture media input.(**Fig. 3a**) Additionally, plasma (~1.5 ml) was run through our TENPO device, and EVs were isolated using GluR2+. (**Fig. 3b**) For Western blot, 20 µg of proteins was loaded per lane. Multiple bands observed in the Western blots and the bands that are below the expected size may be degradation products. Those larger are most likely cross-reacting proteins or possible complexes of the proteins not fully denatured (e.g. CD9 shows a band at approximately double the size of the monomer, suggesting possible dimers). Many reports do not show the existence of these extra bands since the Western blots are cut to show only the expected band, but multiple bands are shown on some of the product sheets for the antibodies purchased (e.g. Anti-ALIX antibody, ab117600, Abcam)

Subtask 5: Isolation of RNA cargo from EVs.

We isolated RNA from the EVs captured by TENPO using the Total Exosomal RNA Isolation Kit (Life Technologies). Then, we characterized the RNA quantity using Qubit (Life Technologies). The quality of the RNA was analyzed using a BioAnalyzer using a DNA 1000 chip. For size selection, AMPure XP beads were used (Beckman Coulter). 140–150 bp sizes were selected using the beads and the sizes were confirmed by the BioAnalyzer using High Sensitivity Chip. For downstream sequencing, the RNA-seq libraries will be pooled together and the final concentration quantified using a KAPA Library Quantification Kit (KAPA Biosystems). The libraries are sequenced using a NextSeq 500/550 kit (FC-404-2005, Illumina) on a NextSeq500 (75 base pair length).

Subtask 6: Develop a droplet detection technology to measure at least three colors allowing ratiometric, calibration free use and expanded multiplexing.

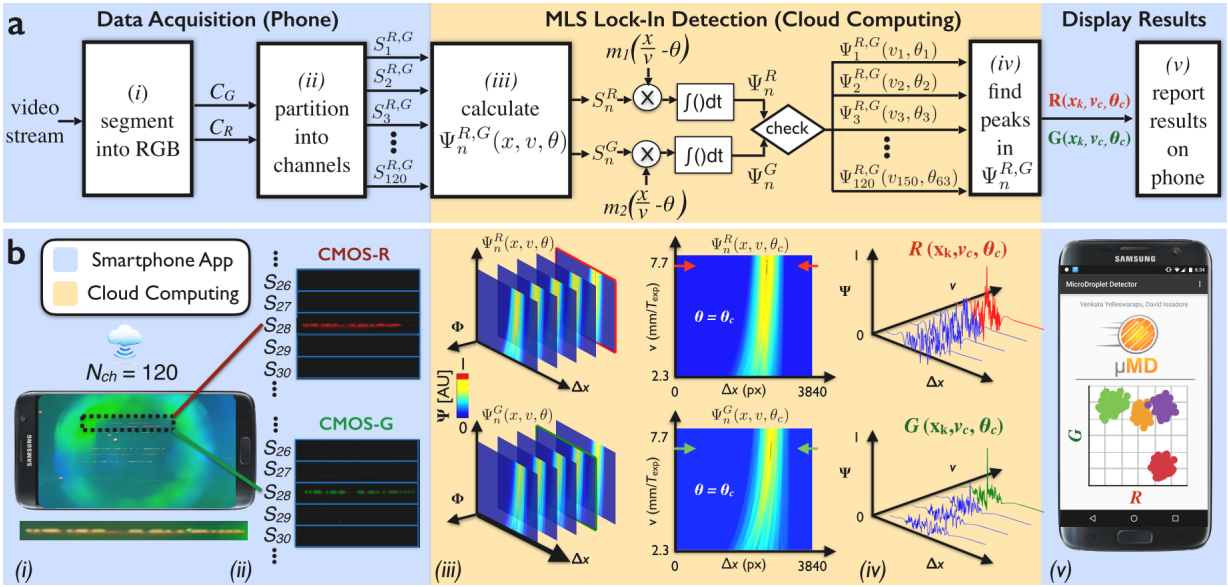

Fig. 4. Software Workflow for multiplexed bead detection. a. Workflow for detecting droplets, with lock-in detection scheme that scans for the emitted fluorescence as a function of droplet velocity and phase at which the excitation pattern starts. **b.** Sample workflow for a single droplet that contains two different fluorescent dyes. Images from the video are partitioned into 1d vectors (*ii*). We generate a 3d correlation matrix with all phases and velocities for a droplet (*ii*), and select the optimal correlation for each MLS (*iv*). From these results, we use the location of the correlation peaks to determine which population a droplet belongs to. The cell phone records the video, while the data is processed using cloud computing, and returns the results on the Android using Matlab Mobile and a custom app that we developed.

Building on prior work, we show that multiple fluorescent dyes can be detected in each individual droplet by using multiple LEDs, each encoded with a unique maximum length sequence (MLS) that can be specifically readout using a correlation based detection, borrowing techniques from the telecommunications industry. (**Fig. 4a**) In this paper, we experimentally demonstrate a two-color system wherein we can detect up to three separate molecular targets in each droplet. (**Fig. 4b,c**)

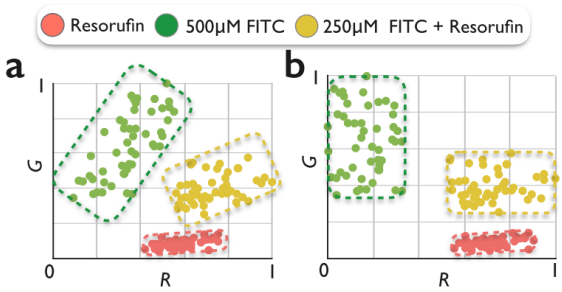

Fig. 5. Experimental Verification of multiplexed bead detection. a. Classification of Resorfuin, FITC, and mixed droplet populations. **b.** Linear transformation correcting for the crosstalk of the FITC droplets results in tighter scatter groups.

We analyzed the computed R and G spread of the scatter for each population and showed a sample of N = 50 droplets (**Fig. 5a**). The scatterplot shows that we can separate all three populations. We used this measurement to correct for crosstalk by a linear transformation where the coefficient of correction matched the calculated crosstalk (**Fig. 5b**).

Major Task 2: In Vitro Biomarker Selection

Subtask 1: To screen for surface marker candidates we will use multiple cell-culture based stretch models of injury including models using nearly pure cultures of neurons, astrocytes, and blood-brain-barrier. We will identify identify surface markers unique to each cell type, and RNA markers with high differential expression between injured and control (sham) state.

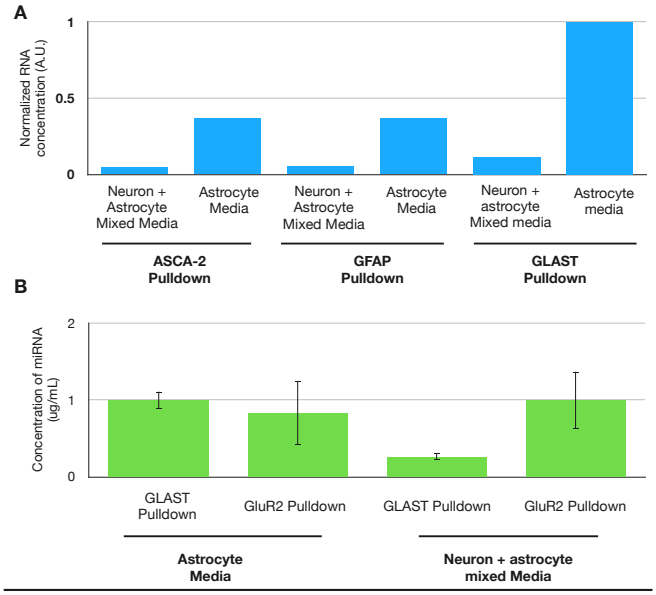

Fig. 6. Experimental Verification of multi-tissue specific pulldown of EVs. a. GLAST outperforms other astrocyte-specific markers in capture of astroglial EVs using the TENPO platform. **b.** GluR2 isolates EVs from both neuron and astroglia, while GLAST is specific for astroglial EVs.

Exosomes can be isolated based on a variety of surface makers, including those that isolate all exosomes (e.g. CD9, CD63, CD81) and those that are brain specific (GluR2, GLAST). In this sub-aim, we identified GluR2 and GLAST as a set of surface markers to specifically isolate exosomes from multiple cell types from the brain to enable the exosome based diagnostic to capture an increasingly comprehensive picture of the injured and recovering brain.(**Fig. 6a,b**) To screen for surface marker candidates we used multiple cell-culture based models of injury including models for neurons and astrocytes, and are continuing this work by inspecting both brain endothelial cells and models for the blood-brain-barrier.

Major Task 3: Pilot Clinical / Porcine Evaluation

Subtask 1: We will isolate multiple extracellular vesicle (EV) subpopulations from injured patients and healthy controls using the TENPO from *N* = 20 injured subjects and *N* = 20 controls.

This task has been completed.

Subtask 2: We will sequence EV isolated from *N* = 40 banked serum samples from a porcine injury model

This work is still in progress.

What opportunities for training and professional development has the project provided?

All students and post-docs in this study have been encouraged to share their work at national and international meetings, and have done so at Neurotrauma, Gordon Conference on Extracellular Vesicles, Keystone on exosomes, BMES, Pitt Con, and at meetings of the International Society of Extracellular Vesicles.

How were the results disseminated to communities of interest?

Kryshawna Beard, David Issadore, and Dave Meaney annually attend Mind Your Brain, an event to share research in brain injury with survivors of traumatic brain injury, held annually at UPenn.

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

In the next reporting period we will complete the final unfinished tasks in Phase 1, and begin work on Phase 2, namely transitioning to work on our pig model for brain injury.

4. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project?

The accurate diagnosis and clinical management of traumatic brain injury (TBI) is currently limited by the lack of accessible molecular biomarkers that reflect the complex pathology of the brain following an injury. To address this challenge, we are developing a microchip diagnostic that can characterize TBI more comprehensively using the RNA found in brain-derived extracellular vesicles (EVs). Our approach measures a panel of EV RNA found in brain derived EVs, processed with machine learning algorithms to capture the state of the injured and recovering brain. Our diagnostic combines surface marker-specific nanomagnetic isolation of brain-derived EVs, biomarker discovery using RNA sequencing, and machine learning processing of the EV miRNA cargo to minimally invasively measure the state of TBI. This approach, which can detect signatures of injury that persist across a variety of injury types and individual responses to injury, more accurately reflects the heterogeneity of human TBI injury and recovery than conventional diagnostics, opening new opportunities to improve treatment of traumatic brain injuries.

What was the impact on other disciplines?

In addition to its intended use in traumatic brain injury, the technology and approaches that we are developing can have applications in a broad range of medical and biological applications. Because EVs are emitted by almost all cells, this approach can be applied to the early diagnosis of cancer, treatment guidance for a wide range of diseases and disorders, and for the diagnosis of infectious diseases, for example.

What was the impact on technology transfer?

The research associated with this grant has lead to the formation of a spin-out company from our lab, Chip Diagnostics. This company has secured venture capital funding and has licensed intellectual property from University of Pennsylvania.

What was the impact on society beyond science and technology?

This research is poised to fundamentally change the way that traumatic brain injuries are clinically managed. For the millions of individuals, and their loved ones, who are afflicted annually by TBI and its longterm consequences, this research has the potential to provide clarity to them and their healthcare providers on their injury, their recovery, and potential pathways towards recovery.

5. **CHANGES/PROBLEMS:**

There were slight delays in our research associated with COVID related shutdown of our lab in the Spring of 2020. Our lab is now operational and we are rapidly getting back on schedule, finishing the sequencing experiments of phase 1.

Actual or anticipated problems or delays and actions or plans to resolve them

There were slight delays in our research associated with COVID related shutdown of our lab in the Spring of 2020. Our lab is now operational and we are rapidly getting back on schedule, finishing the sequencing experiments of phase 1.

Changes that had a significant impact on expenditures

There was an initial delay in hiring of a post-doc into our group. Additionally, one of the students working on this project (Yasemin Atiyas) received a Department of Defense NDSEG fellowship, which covers her stipend and her tuition. We are actively hiring to account for these changes to our budgeted spending.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. **PRODUCTS:**

- **Publications, conference papers, and presentations**

N. Shah, V. Iyer, Z. Gao, Z. Zhang, V. Yelleswarapu, F. Aflatouni, A.T.C. Johnson, and D. Issadore, Graphene micro-Hall sensors for the In-flow detection of rare cells, Submitted, 2020. Fed support acknowledged.

D. Issadore, V. Iyer, Z. Yang, J. Ko, R. Weissleder, Advancing Microfluidic Diagnostic Chips for Clinical Use, Submitted, 2020. Fed support acknowledged.

S. Muraoka, A. M. DeLeo, H. Tatebe, Z. Yang, Y. K. Wang, K. Yukawa-Takamatsu, Y. You , S. Ikezu , T. Tokuda , D. Issadore , R. A. Stern, T. Ikezu, Protein profiling of biomarkers in extracellular vesicles isolated from plasma of former National Football League players at risk for chronic traumatic encephalopathy, Submitted, 2020. Fed support acknowledged.

K. Beard, D. F. Meaney, D. Issadore, Clinical applications of extracellular vesicles in the diagnosis and treatment of traumatic brain injury, Journal of Neurotrauma, doi: 10.1089/neu.2020.6990, 2020. Fed support acknowledged.

S. Muraoka, A.M. DeLeo, M.K. Sethi, K. Yukawa-Takamatsu, Z. Yang, J. Ko, J. D. Hogan, Z. Ruan,Y. You, Y. K.Wang, M. Medalla, S. Ikezu, W. Xia1, S. Gorantla, H. E. Gendelman, D. Issadore, J. Zaia, T. Ikezu, Proteomic Profiling and Biological Characterization of Extracellular Vesicles Isolated from Human Alzheimer's Disease Brain Tissues, Alzheimer's & Dementia: The Journal of the Alzheimer's Association(BioRxiv), 2020. Fed support acknowledged.

H. Shen, T. Liu, J. Cui, P. Borole, A. Benjamin, K. Kording, D. Issadore, A Web-based Automated Machine Learning Platform to Analyze Liquid Biopsy Data, Lab on a Chip, https://doi.org/10.1039/D0LC00096E, 2020. Fed support acknowledged.

Books or other non-periodical, one-time publications. *R*

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

- **Website(s) or other Internet site(s)**

Issadore Lab Website: http://issadore.seas.upenn.edu/

- **Technologies or techniques**

The Track Etched Magnetic Nanopopre (TENPO) technology developed as part of this grant has been patented, and is now being commercialized by a spin-out company from our lab Chip Diagnostics.

- **Inventions, patent applications, and/or licenses**

D. Issadore, M. Muluneh, Magnetic Apparatus and Methods for Analyzing the Output of Microfluidic Devices, US Patent Issued - 10,473,590, 2019. Licensed to Chip Diagnostics.

D. Issadore, M. Muluneh, Magnetic Separation Filters and Microfluidic Devices, US Patent Issued - 10,335, 789, 2019. Licensed to Chip Diagnostics.

D. Issadore, M. Muluneh, Magnetic Apparatus and Method for Manufacturing a Microfluidic Device Filters and Microfluidic Devices, US Patent Issued - 10,632,462, 2020. Licensed to Chip Diagnostics.

E. Carpenter, D. Issadore, B. Stanger, Z. Yang, A Blood Based Multi-Analye Liquid Biopsy Approach for Diagnosis of Pancreatic Adenocarcinoma and Detection of Occult Meastases, Patent Filed - 62/982,254, 2020. Licensed to Chip Diagnostics.

D. Issadore, D. Lee, S. Yadavali, Silicon Chip Having Multi-Zone Through Silicon Vias and Method of Manufacturing The Same, Provisional Patent Filed - PCT/US2020/015684, 2020.

D. Issadore, D. Lee, S. Yadavali, Large Scale Microdroplet Generation Apparatus and Methods of Manufacturing Thereof, Patent Filed - 16/062,724, 2020.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

N*ame:*	*David Issadore*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-5461-8653*
Nearest person month worked:	*2.5*
Contribution to Project:	*Prof. Issadore has contributed to overseeing all aspects of the proposal, but has particularly focused on the technology development aspects..*
N*ame:*	*Dave Meaney*
Project Role:	*Co-PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-0954-4122*
Nearest person month worked:	*1*
Contribution to Project:	*Prof. Meaney has contributed to overseeing all aspects of the proposal, but has particularly focused on the biomarker discovery and porcine model development aspects.*
N*ame:*	*Ramon Diaz-Arrastia*
Project Role:	*Co-PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0001-6051-3594*
Nearest person month worked:	*1*
Contribution to Project:	*Prof. Diaz-Arrastia has contributed to overseeing all aspects of the proposal, but has particularly focused on n the biomarker discovery and the clinical translation aspects.*
N*ame:*	*Danielle Sandsmark*
Project Role:	*Co-I*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-1586-6961*
Nearest person month worked:	*1.8*
Contribution to Project:	*Prof. Sandsmark has contributed to overseeing all aspects of the proposal, but has particularly focused on n the biomarker discovery and the clinical translation aspects.*

N*ame:*	*Yasemin Atiyas*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*6*
Contribution to Project:	*Yasemin has contributed mainly to the fluorescence droplet detection aspects of this work.*
Funding Support:	*Yasemin is now supported by an NDSEG fellowship*

N*ame:*	*Hanfei Shen*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*6*
Contribution to Project:	*Hanfei has contributed mainly to the extracellular vesicle isolation aspects of this project.*

N*ame:*	*Stephanie Yang*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*12*
Contribution to Project:	*Stephanie has contributed mainly to the fluorescence droplet detection and droplet PCR aspects of this work.*

N*ame:*	*Zijian Yang*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*6*
Contribution to Project:	*ZJ has contributed mainly to the fluorescence droplet detection and extracellular vesicle isolation aspects of this work.*

N*ame:*	*Andrew Lin*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*6*
Contribution to Project:	*Andrew has contributed mainly to the extracellular vesicle isolation aspects of this project.*

N*ame:*	*Sagar Yadavali*
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*2*
Contribution to Project:	*Sagar has contributed mainly to the high throughput droplet generation aspects of this project.*

N*ame:* *Kryshawna Beard*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: *6*
Contribution to Project: *Kryshawna has contributed mainly to the extracellular vesicle isolation aspects of this project.*
Funding Support: *Kryshawna is now supported by an NIH training fellowship*

N*ame:* *Cillian Lynch*
Project Role: *Investigator*
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: *1*
Contribution to Project: *Dr. Lynch is the Laboratory Manager working with Drs. Diaz-Arrastia and Sandmark. He has managed the inventory of human biological fluids, and has carried out assays for brain-injury related biomarkers.*

N*ame:* *Leroy Wesley*
Project Role: *Research Assistant*
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: *4*
Contribution to Project: *Mr. Wesley is a research assistant working with Drs. Diaz-Arrastia and Sandsmark. He has been involved in recruiting TBI subjects and well as healthy control participants, processing and storing biological samples.*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Nothing to report.

8. **SPECIAL REPORTING REQUIREMENTS**

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. **APPENDICES:**