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TITLE: Biofidelic Three-Dimensional Brain Surrogate
Models of mTBI-Induced Alzheimer's Disease Pathology

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 None

14. ABSTRACT
 The vast complexity of the brain, with its hundred billion neurons and supporting cells as well as hundreds of trillion connections, poses a tremendous roadblock for scientists to understand the working of the brain on molecular, cellular, or circuit levels. Defining the genetic programs that drive neural function, the cell-type specific contributions to neural circuit working, the mapping of connectivity patterns within and between individual networks, and elucidating the mechanisms of disease present only a few examples of the challenges. Novel approaches and technologies are needed to complement and advance the state-of-the art *in vivo*, *ex vivo*, and *in vitro* approaches to study brain physiology and pathology. Here, we are proposing to bioengineering a validated *in vitro* 3-dimensional (3D) brain surrogate mTBI/AD model built of primary mouse neurons. Our research proposal builds upon the shock wave model of mTBI, which postulates that mTBI is caused by the primary shock wave from a blast that penetrates through the skull and traverses the brain. We will use this to elucidate the mechanisms leading to open field blast explosives induced mTBI and its relationship to Alzheimer's disease, including discovery by proteomic, genomic, and *in vivo* analysis of mice of new mTBI/AD biomarkers and disease pathways.

15. SUBJECT TERMS
 Engineering a validated *in vitro* 3-dimensional (3D) brain surrogate mTBI/AD model

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INTRODUCTION

Three-dimensional (3D) surrogate models that mimic the actual geometry, composition, and function of neural circuits present the most physiologically relevant *in vitro* system to study brain physiology in health and disease. However, contrary to other research fields (e.g., immunology and cancer), neuroscience still lacks an *in vitro* setting to examine cellular function in 3D in densely wired, heterogeneous tissues. Novel approaches and technologies are urgently needed to complement and advance the state-of-the-art *in vivo*, *ex vivo*, and *in vitro* approaches as well as to study brain physiology and pathology. Here, we are proposing to bioengineer a validated *in vitro* 3-dimensional (3D) brain surrogate mTBI/AD model built of primary mouse neurons. Our research proposal builds upon the shock wave model of mTBI, which postulates that mTBI is caused by the primary shock wave from a blast that penetrates through the skull and traverses the brain. We will use this to elucidate the mechanisms leading to open field blast explosives induced mTBI and its relationship to Alzheimer's disease, including discovery by proteomic, genomic, and *in vivo* analysis of mice of new mTBI/AD biomarkers and disease pathways.

KEYWORDS

3-D Brain Models, Microphysiological Models, Alzheimer's Disease, Traumatic Brain Injury, Neural Tissues

1. Accomplishments

What were the major goals of the project?

The objective of our research is to bioengineer a validated *in vitro* 3-dimensional (3D) brain surrogate mTBI/AD model built of primary neurons. Our research proposal builds upon the shock wave model of mTBI, which postulates that mTBI is caused by the primary shock wave from a blast that penetrates through the skull and traverses the brain. The resulting ultrastructural damage and injuries to nerve cells at molecular, cellular, and brain circuit levels may lead to mTBI/AD pathology. We will use this to elucidate the mechanisms leading to open field blast explosives induced mTBI and its relationship to Alzheimer's disease, including discovery by proteomic, genomic, and *in vivo* analysis of mice of new mTBI/AD biomarkers and disease pathways.

What was accomplished under these goals?

Specific objectives addressed

As specified in Aim 1, Dr. Demirci and his team at Stanford University have worked on fabrication various 3D brain surrogates for long-term culture. At the same time, the property of the hydrogel used for 3-D neuron culture could influence neuron growth and neural stem cell differentiation. This was also studied as a parameter for blast experiment.

As specified in Aim 2, Demirci lab transfer the knowhow and the materials to Gu lab for his team at the University of Missouri. Dr. Gu and his team at the University of Missouri have worked on the 3-D neuron cultures in Missouri and blast on the field.

As specified in AIM 3, Dr. Gu and his team at the University of Missouri have worked on establishing the relevance to mTBI/AD pathology in animal models exposed to primary shockwaves induced by open-field explosive blasts.

As specified in **Subtask 9 (Aim3)**, our team at the University of Missouri (UM) has worked on the logistic aspects proposed for the development of our experimental systems for modeling of combat blasts using military relevant open field explosive damage paradigms.

Major activities

1. Animal protocols for isolating neurons at the Stanford University are approved.
2. Dr. Demirci and his team have performed experiments to demonstrate the ability to grow primary neurons isolated from mouse in 3D *in vitro* cultures using different biomaterials such as Matrigel, Fibrin and GelMA (methacrylated gelatin) hydrogels. The viability assay results demonstrated that utilizing GelMA is better for long-term 3-D culture of primary neurons compared to Matrigel.
3. Dr. Demirci and his team have performed long-term experiments to demonstrate the ability of mouse primary neurons to grow in 3D in GelMA hydrogel (Figure 1). They further quantified the length of functional axons, dendrites and the number of synaptic vesicles in this network.

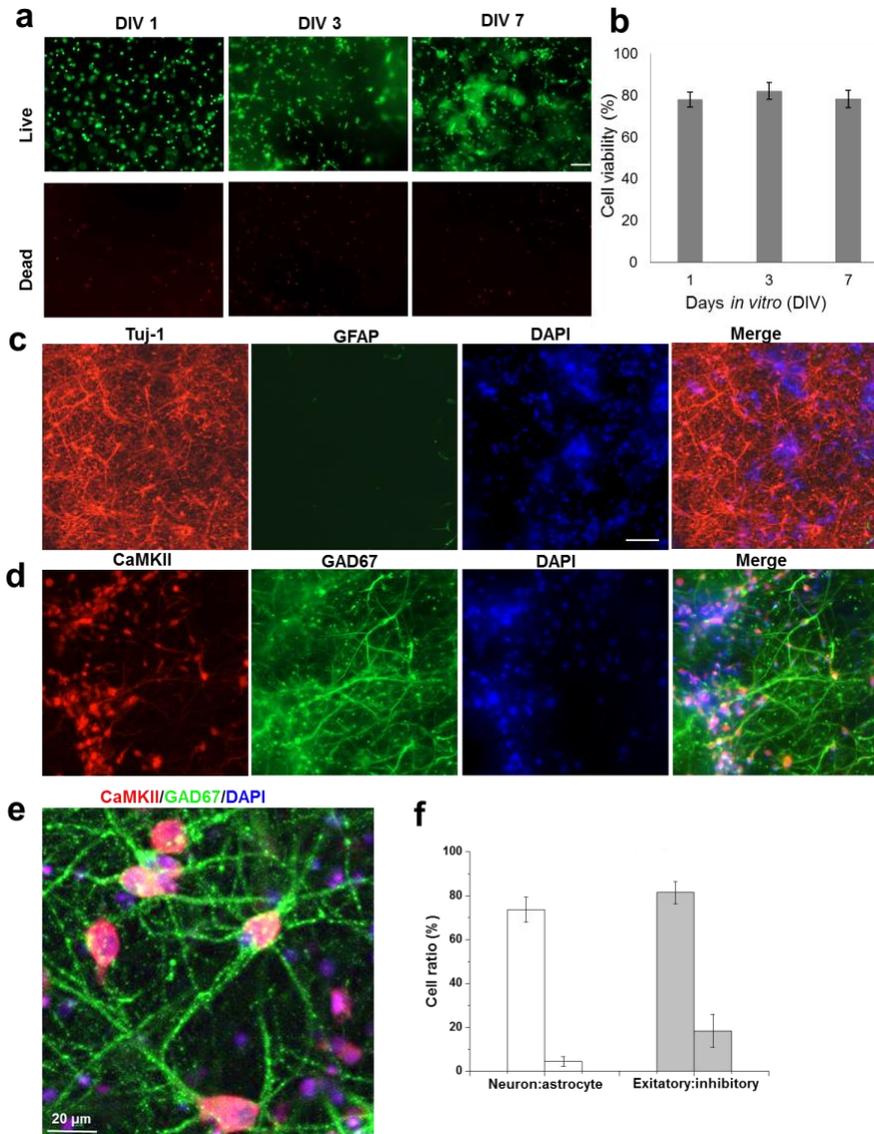


Figure 2 (a) Images and (b) quantification of neuronal cell viability in GelMA 3-D mini-gel using calcein-AM (green, live) and ethidium homodimer-1 (red, dead). Scale bar indicates 100 μ m. Standard deviation was calculated for $n = 3$ with significance $p < 0.05$. Samples were not significantly different according to the one way ANOVA analysis. (c) Neuro-astro-network of neuronal cell cultures in 3-D GelMA mini-gel. “Red” indicates anti-beta-tubulin staining, “green” indicates anti-GFAP staining and “blue” indicates DAPI staining. (d) Immunostaining showing excitatory and inhibitory neurons in 3-D milli-block. Anti-GAD67 indicates inhibitory neurons (green) and anti-CaMKII indicates excitatory neurons (red). Scale bar indicates 100 μ m if not specifically labeled.

- To study neuronal network activity and their reaction to stimuli, three-week-old 3D neuronal tissues were treated with a non-competitive protein-conjugated GABA_A receptor antagonist, Picrotoxin (PTx), to block inhibition and increase network activity. We observed a steep increase of fluorescent intensity of calcium signal upon PTx adding (Figure 2a), and the spiking frequency and correlation coefficient also increased, however, there was no significant difference before and after PTx treatment (Figure 2b,c). Glutamate is a neurotransmitter which

widely exists in most excitatory neurons as well as the glial cells. Agreeing with previous reports, the addition of glutamate caused a sharp rise of fluorescent intensity of calcium signal (Figure 2a). The pulsating curves (Figure 2d) were further turned into a heatmap with intensity varied over time for easier comparison of frequency spikes (Figure 2e). The dynamics consisting of network bursts lead to the conclusion that a functional and active neuronal network was formed when culturing neuronal cells in 3-D GeIMA hydrogels over the course of three weeks.

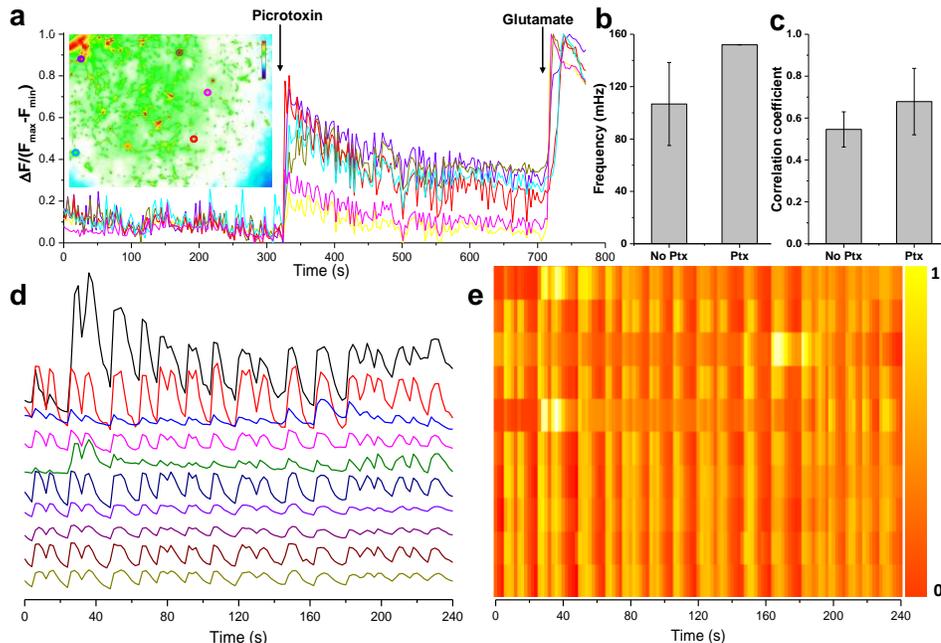


Figure 2 (a) Ca^{2+} intensity traces of 5 neurons marked in the image at the top left of the graph. PicROTOXIN and Glutamate was added at 330 s and 720 s, respectively. Calcium Oscillation frequency and correlation coefficient were analysis and showed in (b). More Ca^{2+} (c) traces ($\Delta F/F_0$) and (d) time-series heatmap of ($\Delta F/(F_{\max}-F_{\min})$) calculated from 10 individual neurons in milli-block which were recorded in Video. The amplitude of the calcium oscillation is indicated as a color intensity in (b), the lighter color indicate higher fluorescent intensity. Traces are displayed over the course of 240 seconds.

5. Shipping condition for 3D culture neurons were tested in Demirci Lab, for DIV3, keeping at 37 °C for 24 hrs without CO_2 supply can maintain 70% cell viability but for DIV7 neurons, a large percentage of cells were dead after 24 hrs.
6. Dr. Gu was able to repeat the 3-D neuronal cultures and grow them in university of Missouri to prepare these cultures for blast experiments.
7. Blast experiments were performed with the 3-D cultures for multiple various conditions and the post-blast neuronal 3D cultures were tested for viability. This led to the conclusion that the blast conditions have to be further optimized for 3-D cultures. We learned that the animal blast conditions and the 3-D culture blast conditions need to be significantly different given the lack of a skull to protect the neurons.
8. We discovered at 3 m distance in 30 days after blast, 1) Axons are less compacted; 2) Some myelin sheath layers were broken and myelin was deteriorated on cortex; 3) Some mitochondria are damaged.

9. Cortical brain tissues were prepared for the proteomic study. Liquid chromatography-mass spectrometry (LC-MS/MS) was used to analyze brain tissue labeled with isobaric mass tags for relative protein quantification. We demonstrated both phosphorylated tau (p-tau) and the ratio of p-tau/tau were significantly increased in cortical brains of mice after exposure to low-intensity open-field blast. The results from the proteomics and bioinformatic analysis illustrated the alterations of axonal and synaptic proteins in related pathways, suggesting a potential axonal damage caused by blast-induced mTBI. Among altered proteins found in brains suffering blast, microtubule-associated protein 1B, stathmin, proteom neurofilaments, actin binding proteins, myelin basic protein, calcium/calmodulin-dependent protein kinase, and synaptotagmin I were representative ones involved in altered pathways elicited by mTBI.
10. Our recently published report by Song et al (2018) demonstrated striking dynamic changes in a total of 2216 proteins and 459 phosphorylated proteins at vary time points after blast. Disruption of key canonical pathways included evidence of mitochondrial dysfunction, oxidative stress, axonal/cytoskeletal/synaptic dysregulation, and neurodegeneration (Figure 3). With observations of proteomic changes, we found low-intensity blast (LIB)-induced oxidative stress associated with mitochondrial dysfunction. These dysfunctions included impaired fission-fusion dynamics, diminished mitophagy, decreased oxidative phosphorylation, and compensated respiration-relevant enzyme activities.

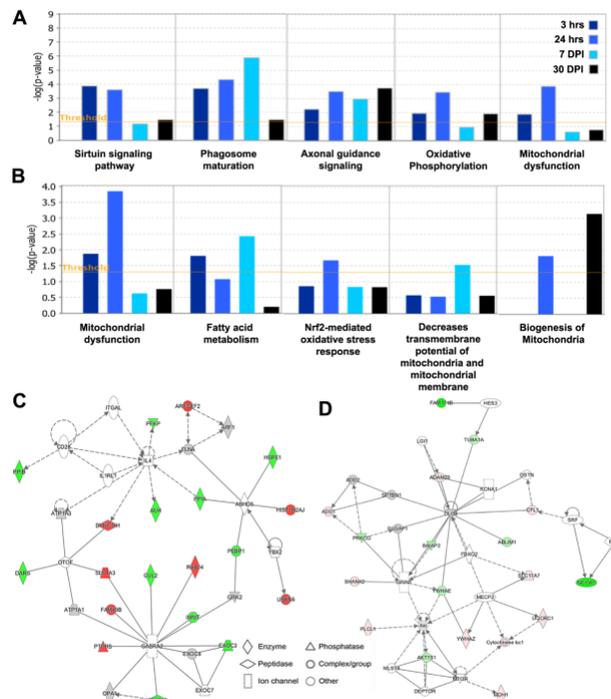


Figure 3. LIB effects at different time on alterations of canonical pathways, toxic annotation, and disease/function networks. (A) Top five canonical pathways predicted by an IPA analysis of the differentially expressed global- and phospho-proteins affected by the low-intensity blast exposure at different time points (color coded). (B) Top five toxic lists predicted by IPA. The canonical pathways and toxic list annotations were ranked according to the $-\log(P)$ value. A ratio (height) indicates the number of proteins that were differentially expressed in each pathway or list over the total numbers of proteins in that specific pathway. (C) The top disease/function network in global-

proteome is associated with cellular development, cellular growth and proliferation, cellular movement, and cellular assembly and organization corresponding to the low-intensity blast. (D) The top disease/function network in phospho-proteome is associated with neurological disease, organismal injury and abnormalities, cell-to-cell signaling and interaction, and cell morphology corresponding to the low-intensity blast. The identified genes involved in the networks were displayed in red (up-regulation) and green (down-regulation) color. The color intensity indicates the degree of regulation. Solid lines in the network imply direct interactions between genes, and dashed lines indicate indirect interactions. Geometric shapes represent different general functional families of gene regulation (diamond for enzyme, oval for transcription regulator, trapezoid for transporter, inverted triangle for kinase, double circle for complex/group, and circle for others).

11. Our findings on loss of mitochondrial fission and fusion proteins and consequently compromised activities suggest a causal role in the pathogenesis of LIB brain injury and possibly initiation of later neurodegeneration (Figure 4).

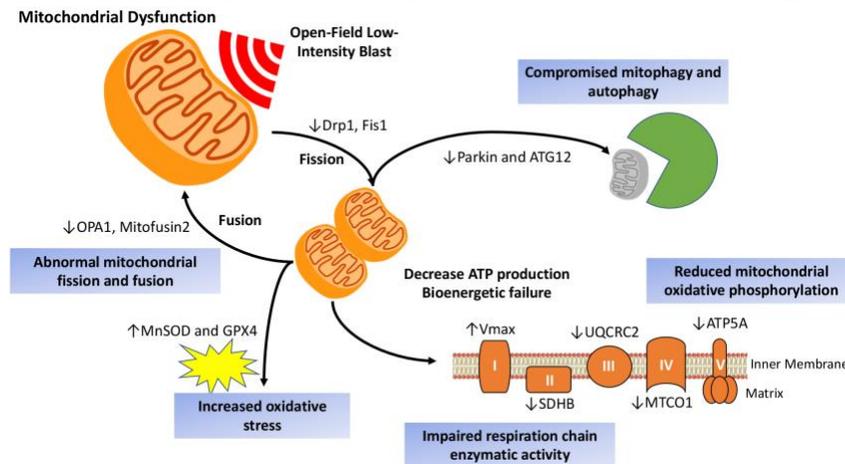


Figure 4. A scheme depicting effects of open-field low-intensity primary blast on mitochondrial dysfunction and canonical pathways. Quantitative proteomics and biochemical analysis reveal the mitochondrial dysfunction following low-intensity primary blast. We identified blast-induced mitochondrial damages associated with impaired fission-fusion dynamics, diminished mitophagy, increased oxidative stress, decreased oxidative phosphorylation, and compensated respiration activity.

12. We demonstrated ultrastructural changes in the brain in the mouse model subjected to open-field blast exposure at 46.6 kPa. To further understand the molecular mechanisms underlying changes due to low-intensity blast, we have collected cortical tissues from mice and applied quantitative proteomics and bioinformatics analysis to determine the global- and phospho-proteome at 30 DPI after blast. Low-intensity blast induced differentially expression of 173 global-proteins point at 30 DPI vs sham.
13. Myelin sheath in different brain regions were surveyed by TEM in the sham control and the mice at 3-m away from a 350-g C4 blast exposure. We observed myelin sheath defects identified in the brain in mice exposed to such low-intensity blast (Figure 5). Our observations and quantitation suggested there were some degree recovery of myelin sheaths over time in mice after low-intensity open-field blast exposure.

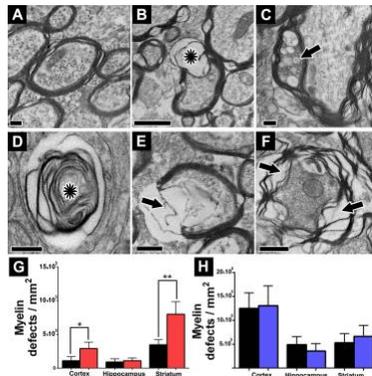


Figure 5. Myelin sheath defects identified in different brain regions after open field blast mild TBI. (A) Representative image of a normal myelinated sheath which appear as a thick, electron-dense and tightly wrapped around the axon in a sham mouse cortex. (B) “Myelin balloon” is characterized by bulges of split myelin layers indicated by the asterisk. (C) Dense “redundant” myelin sheath degeneration represented by pockets of vacuoles and dense cytoplasm within the myelin layers indicated by the asterisks. (D) Myelin lamellation with a collapsed inner core leading to the so-called “myelin onion”. (E) Myelinated axons with extended area of “disrupted” split myelin layers are shown by the arrows. (F) Myelin “detachment” is loosely wrapped abnormal myelin sheaths leaving a hypodense space between the axon membrane and the inner myelin layers indicated by the asterisk. (G) Comparisons of the myelin sheath defects at the 3-m blast mice vs. negative sham control. A significantly higher number of myelin sheath defects were observed in 3-m blast mice (red bars) at 7 DPI compared to sham control (black bars). One tailed paired t-test; * $P < 0.05$; **, $P < 0.01$; $n = 4-5$. (H) However, there is not statistically significant difference at 30 DPI for the 3-m blast mice and sham control. Scale bar = $0.5 \mu\text{m}$ (A, C, E); $1 \mu\text{m}$ (B, D, F).

14. Cell cultures have been performed in Columbia. We have tried two types of 3-D culture conditions (Gelma and fibrinogen) and used 2D cultures as control for monitoring cell quality in Missouri.
15. Missouri team has worked closely with Stanford group to test 3D cultures in Missouri, modifying the protocol and looking for the optimal culture conditions.
16. Transferring 3D cultured cells between Columbia (culture location) and Rolla (blast location) would not effort the culture condition.
17. Tested blast conditions on 3D cultures. Cells were died after expose to C4 350 g (similar condition as on mice, Figure 6). This is likely due to the geometrical and anatomical difference between the ex vivo cortical cell 3D cultures and in vivo mouse brain under such open-field blast exposure. Here we reduced the blast intensity with C4 at 80 g at variance distances away. Results showed that blast at 7m distance away [1.2 PSI (8.3 kPa) peak overpressure with 2.6-ms positive phase duration, and 1.6PSI*ms impulse] induced cell deaths at 1 day post injury (DPI). Sham group, cells were in the same condition with experimental blast cells, but without blast exposure, were growing well. We also tested the culture conditions with or without transportation involvement. There is no significate difference. It indicated that cells are not affected by transportation.

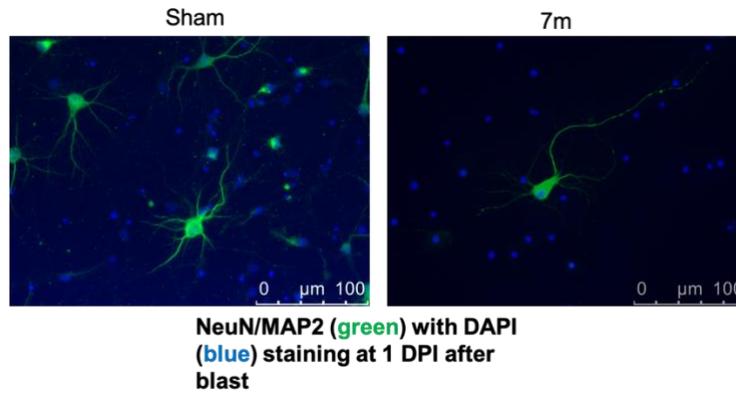


Figure 6. NeuN/MAP2 staining showing that blast caused cell death compared to sham group.

18. The intensity was reduced again (C4 was 20 g). The peak pressure at 0.45 psi induced cell death in one day after blast.
19. Based on the previous data, we continue using fibrinogen 3D cultures to study neural stem cell differentiation.

Describe the Regulatory Protocol and Activity Status (if applicable).

(c) Animal Use Regulatory Protocols

TOTAL PROTOCOL(S): 3

PROTOCOL (1 of 3 total): Stanford University

Protocol [ACURO Assigned Number]: **AZ140109**

Title: Isolation of Mouse Brain Cells

Target required for statistical significance: 65 for the duration of the grant

Target approved for statistical significance: 65 for the duration of the grant

Submitted to: Sheron Westbrook, RVT, RLATG, CPIA, SLAVT, Animal Use Review Specialist

Approved by: USAMRMC Animal Care and Use Review Office (ACURO)

Status: approved and current

PROTOCOL (2 of 3 total): University of Missouri

Protocol [ACURO Assigned Number]: **AZ140109.02**

Title: *Development of an Open-Field Blast-Induced Brain Injury Model for Mechanistic Studies*

Target required for statistical significance: 36 for the duration of the grant

Target approved for statistical significance:

Submitted to: Sheron Westbrook, RVT, RLATG, CPIA, SLAVT, Animal Use Review Specialist

Approved by: USAMRMC Animal Care and Use Review Office (ACURO)

Status: approved and current

PROTOCOL (3 of 3 total): University of Missouri

Protocol [ACURO Assigned Number]: **AZ140109.03**

Title: Primary Culture in Rats and Mice

Target required for statistical significance: 15 timed pregnant mice for the duration of the grant

Target approved for statistical significance:

Submitted to: Sheron Westbrook, RVT, RLATG, CPIA, SLAVT, Animal Use Review Specialist

Approved by: USAMRMC Animal Care and Use Review Office (ACURO)

Status: approved and current

What do you plan to do during the next reporting period to accomplish the goals and objectives?

We aim to test open-field blast conditions towards to **Aim 1, SubTask4-5** Generation of a militarily relevant blast environment to create 3D brain surrogate models of mTBI/AD And study ultrastructure damage and the relative biomarker by qPCR, western blot, immunofluorescence microscopy methods. We will also use genomics and proteomics technology to analyze the disease pathologic pathway.

2. Impact: Nothing to report at this stage.

3. Products: Nothing to report at this stage.

4. Participants & Other Collaborating Organizations:

Stanford University personnel:

Name: Utkan Demirci
Project Role: PI
Research Identifier: utkandemirci (NIH agency login)
Month worked: 3 days
Contribution to Project: Supervision of project at Stanford.

Name: Loza Tadesse
Project Role: Graduate Student
Research Identifier: none
Month worked: 1 months
Contribution to Project: Materials engineering, biomaterial modification, scaffold/hydrogel engineering, microscopy and immunostaining.

Name: Shreya Deshmuch
Project Role: Graduate Student
Research Identifier: none
Month worked: 3 months
Contribution to Project: Isolation of neural stem cells and cultures both in 2D and 3D, analysis of cultures via immunostaining

University of Missouri Personnel:

Name: Zezong Gu
Project Role: co-PI
Research Identifier: zegunih1 (NIH agency login)
Month worked: 2 months
Contribution to Project:

Oversee and supervising the activity on MU including the sites on Columbia and blast site on Rolla, including but not limited on , experimental design and performance, analyzing data, writing reports and manuscript.

Nearest person: Jiankun Cui
Project Role: Senior Personnel
Research Identifier: none
Month worked: 2 months

Contribution to Project:

With her expertise, assisted Dr. Gu including but not limited on, experimental design/planning and performance, writing the U of Missouri animal protocols, working with other lab members on the tissue preparation for TEM and other studies, cell cultures, analyzing data, writing reports and manuscript.

Name: Hailong Song
Project Role: Graduate Student
Research Identifier: none
Month worked: 5 months

Contribution to Project:

Involving animal blast experiment in Rolla and taking care mice, cell culture, immunostaining, preparation of brain tissue and sections for EM and other studies, working with MU EM core facility and other collaborators including Stanford on cell culture and others, and analyzing data. He is the major person, working closely with Dr. Cui, performing the experiments in Gu lab

Name: Tina Ndam
Project Role: Lab technician
Research Identifier: none
Month worked: 6 months

Contribution to Project:

Helping immunostaining, preparation of brain tissue and sections for EM, analyzing EM images. Maintain research records, help for general lab maintains.

Name: Landry Konan
Project Role: Graduate student
Research Identifier: none
Month worked: 6 months

Contribution to Project: EM data analysis, manuscripts writing and editing.

Name: 2 other students in Gu lab
Project Role: Medical students
Research Identifier: none
Month worked: 4 months

Contribution to Project: Involving brain preparation and analyzing EM images.

Name: Runting LI
Project Role: Lab technician
Research Identifier: none
Month worked: 1 month
Contribution to Project:
General lab work and assistant PI and others in Gu lab for the project, involving, but not limited, animal care, order supplies, and assistant some experiments.

Person: Catherine E Johnson and her team
Project Role: Engineer
Research Identifier: none
Month worked: 3 months
Contribution to Project:
Dr. Johnson worked closed with Dr. Gu and his team on blast experimental design, blast settings. She also involved manuscript editing.
Under Dr. Johnson's supervision, the team has performed the blast in Rolla and analyzed physical data from blast sitting.

Name: Graham Hubler
Project Role: Consultant
Research Identifier: none
Month worked: 1 months
Contribution to Project: Involving experimental design and planning, data analyzing and project discussion, and manuscript editing.

Name: Ralph G DePalma
Project Role: Consultant
Research Identifier: none
Month worked: 0.5 months
Contribution to Project: participated in experimental design, data analyzing and project discussion, manuscript editing.

5. Changes/Problems

Our highest risk hypothesis was to show the nanoscale damage in the brain is actually present after exposure to blast. This would be a first and a significant finding if we successfully showed this damage with TEM. It took lots of optimization steps and biological investigation. At the end, we showed that our hypothesis had merit and we published these results successfully. This definitely took us longer overall to establish new methods for an unknown nanoscale artifact that we were looking at the nanoscale. It also became more costly than we anticipated, but, at the end, we successfully established that the nanoscale damage is present and can be detected. We shared these exciting results as we confirmed them in our earlier reports.

The second goal was to overall build 3-D neuronal cultures and show that they are both viable and functional in a petri dish using microscale neuronal constructs. We also needed to show that they responded to a stimulus such as toxins and characterize them

for their firings under various conditions to indicate that they are viable and responsible. We also established this and published it recently. Another paper is under review for investigating the functional effects of microscale micro-environment on these 3-D cultures. One finding that we are showing is the size of the 3-D structures has an effect on how neurons talk to each other in response to stimuli. We will publish our initial findings and continue to explore what it means overall. Here we share the draft that is under review.

Third goal was to transfer the knowhow and cultures to Dr. Gu's lab, and repeat the experiments in a different lab setting to confirm our finding with 3-D cultures. We learned that shipping these live 3D structures is not the best way to go due to concerns about diminished viability and functionality. So, we then designed around the problem and established these neuronal cultures at Dr. Gu's lab. They were able to repeat our results and this challenge was overcome. We can culture these living 3-D structures at the University of Missouri that we proposed and built.

Fourth goal was to expose these structures to blast at Dr. Gu's lab. We also performed these experiments last blast season. We realized that we had to change our blast conditions because in absence of a skull that reduces the shock, the 3-D cultures were damaged to a stage, where the cell death was taking over the nanoscale effects that we have been exploring. So, we are now at a stage, where we need to find the correct amount of blast or distance from the blast to have the nanoscale damage acting and observable but without killing or completely destroying the cells. As these challenges come along the way and we address them as we go. So, we explored building a specific cage in order to reduce the blast effects, but we still saw the extreme damage on the 3-D cultured structures.

Overall, we had success showing what we hypothesized around nanoscale damage and building live and functional 3-D neuronal culture systems *in vitro*. We plan to continue to figure out the last risky piece of the project to see if we can mimic the nanoscale damage under culture conditions. We realize that it is an overarching investigation for any 3-D *in vitro* system to see how it will mimic the animal experiments. Overall, we proposed a project that had huge steps forward in our understanding as we put it together. So far we have been successful in mitigating the expected and unexpected challenges of the project and delivering almost all of the goals and we were happy to have proven that the core hypothesis actually turned out to be true.

The remaining step is to explore the blast conditions and reduce impact to a level that we can see the outcomes in 3-D cultures. This last piece has already taken some design changes, but it is massive enough to become the subject of a whole investigation given that there minimal amount of work in this direction in the field. Although this aspect makes this work very innovative, it also comes with its unique challenges making it extremely high-risk and high-return as a project. We asked for a no-cost extension to make it to the next blast season to test some new explosion modalities for the 3-D cultures. We also in parallel continue to characterize and explore the functional aspects of these 3-D cultures under different stimuli and microenvironment. If the culture blast conditions continue to create damage beyond

expected in our next set of experiments, as these systems are mainly optimized for small animal experiments, we will report on our findings with the *in vitro* functional studies at the end of our project and explore new modalities for mechanical damage on the 3-D cultured functional neurons.

6. Special Reporting Requirements: none

7. Appendices:

Meetings:

Stanford University

1. Three-Dimensional Gradients in Isotropic and Anisotropic Mimetic Scaffolds for Regenerative Medicine Applications/Research Presentation, Forefront Research in 3D Disease Cancer Models *in vitro* Screening Technologies (FoReCaST), The tumor microenvironment - 1st Workshop, Porto, Portugal, 2018

Type: Keynote presentation by Dr. Utkan, demirci

2. Guided Self-assembly and Coding of Three-dimensional Living Architectures/Research Presentation, American Association of Dental Research, Fort Lauderdale, FL, 2018

Type: Invited talk by Dr. Utkan, demirci

3. Microfluidic Magnetic and Acoustic Field-based Technologies for 3-D Biofabrication/Research Presentation, The Frontiers of Biomedical Engineering-based Translational Medicine, The 4th International Summer School for Graduate Students Zhejiang University, Zhejiang Sheng, China.

Type: Keynote presentation by Dr. Utkan, demirci

5. Mechanosensing of neurons in 3D brain surrogate. Stanford Bio-X, Stanford, CA, 2017

Type: Poster presentation by Ren, T, Karaca, E., Canadas, R. Demirci, U.*

6. Gradient control and microporosity guidance in 3D structures for regenerative medicine. Stanford Bio-X, Stanford, CA, 2017

Type: Poster presentation by Canadas, R., Tocchio, A., Marques, A., Oliveira, J., Reis, R., Demirci, U.*

7. 3-D geometry and irregular connectivity dictate neuronal firing in frequency domain and synchronization. BMES, Atlanta, GA, 2018

Type: Poster presentation by Tanchen Ren, Bianka Grosshäuser, Kaushik Sridhar, Thomas J.F. Nieland, Alessandro Tocchio, Ute Schepers, Utkan Demirci*

University of Missouri

1. 2017 Military Health System Research Symposium (MHSRS):

Type: poster presentation

Title: The behaviors and neuropathology linked with biophysics in a murine model of open-field blast-induced mild traumatic brain injury

Authors: Hailong Song, Landry Konan, Jiankun Cui, Tina Ndam, Agnes Simonyi, Catherine E. Johnson, Ibolja Cernak, Utkan Demirci, Graham G. Hubler, Ralph G. DePalma, Zezong Gu.

2. 2017 Society for Neuroscience (SfN) Annual Meeting:

Type: poster presentation

Title: The behaviors and neuropathology linked with biophysics in a murine model of open-field blast-induced mild traumatic brain injury

Authors: Hailong Song, Landry Konan, Jiankun Cui, Tina Ndam, Agnes Simonyi, Catherine E. Johnson, Ibolja Cernak, Utkan Demirci, Graham G. Hubler, Ralph G. DePalma, Zezong Gu.

3. 2018 Veterans Affairs (VA) Meeting on “New perspectives on central and peripheral inflammation in traumatic brain injury” at Tampa, FL:

Type: oral presentation by Dr Zezong Gu

Title: Open-field blast injury in mice: Low-intensity primary blast induces ultrastructural brain abnormalities and associated behavioral changes

4. 2018 National Neurotrauma (NSS) Meeting:

Type: poster presentation

Title: Low-intensity primary blast induces nanoscale brain damage and associated behavioral impairments in mice

Authors: Hailong Song, Landry M. Konan, Jiankun Cui, Catherine E. Johnson, Martin Langenderfer, DeAna Grant, Tina Ndam, Tommi White, Utkan Demirci, David R. Mott, Doug Schwer, Graham K. Hubler, Ibolja Cernak, Ralph G. DePalma, Zezong Gu

5. 2018 Big Data Neuroscience Meeting:

Type: poster presentation

Title: Quantitative Proteomic Analysis Reveals Mitochondrial Dysfunction following Low-Intensity Primary Blast Exposure

Authors: Hailong Song, Mei Chen, Chen Chen, Jiankun Cui, Jianlin Cheng, Ralph G. DePalma, Weiming Xia, Zezong Gu

6. KC VA Med Center, Sep 19, 2018

Type: oral presentation by Dr Zezong Gu

Title: To Blast or Not: Matters to Veterans Suffering Mild TBI: Low-intensity primary blast induces brain molecular and ultrastructural abnormalities associated with cognitive deficits

7. 2018 SfN Annual Meeting:

Type: poster presentation

Title: Characterization of mitochondrial damage due to low-intensity primary blast injury

Authors: Hailong Song, Landry M. Konan, Jiankun Cui, Catherine E. Johnson, Martin Langenderfer, DeAna Grant, Bo Yang, Xiaowan Wang, Tommi White, C. Michael Greenlief, Utkan Demirci, Grace Y. Sun, Graham K. Hubler, Russell Swerdlow, Ibolja Cernak, Ralph G. DePalma, Zezong Gu

8. NIA Director's Regional Meeting on the KU Edwards Campus, Kansas City, KA, Nov 1, 2018

Type: oral presentation by Dr Zezong Gu

Title: Mitochondrial dysfunction: A link from blast-induced mild TBI to neurodegeneration and dementia?

Journal publications.

1. Canadas, R., Ren, T., Tocchio, A., Marques, A.P., Oliveira, J.M., Reis, R.L., Demirci, U.*, Tunable anisotropic networks for 3-D oriented neural tissue models. **Biomaterials**, 2018;181:402-414.

2. Canadas, R. F., Ren, T., Marques, A.P., Oliveira, J.M., Reis, R.L., Demirci, U*., Biochemical gradients to generate 3-D heterotypic-like tissues with isotropic and anisotropic architectures. **Advanced Functional Materials** 2018; 0(0): 1804148.

3. Ren, T., Grosshäuser, B., Sridhar, K., Nieland, T., Tocchio, A., Demirci, U., 3-D geometry and irregular connectivity dictate neuronal firing in frequency domain and synchronization. **Biomaterials**, 2018, under review
4. Hailong Song, Landry M. Konan, Jiankun Cui, Catherine E. Johnson, Martin Langenderfer, DeAna Grant, Tina Ndam, Agnes Simonyi, Tommi White, Utkan Demirci, David R. Mott, Doug Schwer, Graham K. Hubler, Ibolja Cernak, Ralph G. DePalma, Zezong Gu. Ultrastructural brain abnormalities and associated behavioral changes in mice after low-intensity blast exposure. **Behavioural Brain Research**. 2018 Jul 16;347:148-157. doi: 10.1016/j.bbr.2018.03.007. Epub 2018 Mar 8. (Cite by 2)
5. Hailong Song, Landry M. Konan, Jiankun Cui, Catherine E. Johnson, Graham K. Hubler, Ralph G. DePalma, Zezong Gu. Nanometer Ultrastructural Brain Damage following Low Intensity Primary Blast Wave Exposure. **Neural Regeneration Research**. 2018;13:1516-9. doi: 10.4103/1673-5374.237110.
6. Mei Chen*, Hailong Song*, Jiankun Cui, Catherine E. Johnson, Graham K. Hubler, Ralph G. DePalma, Zezong Gu*, Weiming Xia*. Proteomic Profiling of Mouse Brains Exposed to Blast-induced Mild Traumatic Brain Injury Reveals Changes in Axonal Proteins and Phosphorylated Tau. **Journal of Alzheimer's Disease**. 2018 Oct 13; doi: 10.3233/JAD-180726
7. Hailong Song, Mei Chen, Chen Chen, Jiankun Cui, Catherine E. Johnson, Jianlin Cheng, Xiaowan Wang, Russell H. Swerdlow, Ralph G. DePalma, Weiming Xia*, Zezong Gu*. Proteomic Analysis and Biochemical Correlates of Mitochondrial Dysfunction following Low-Intensity Primary Blast Exposure. **Journal of Neurotrauma** 2018 Nov 28; <https://doi.org/10.1089/neu.2018.6114>