AWARD NUMBER: W81XWH-15-1-0576

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

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REPORT DATE: DECEMBER 2019

TYPE OF REPORT: FINAL

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE		*Form Approved OMB No. 0704-0188*
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1. REPORT DATE DECEMBER 2019	**2. REPORT TYPE** FINAL	**3. DATES COVERED** 15SEP2015 -14SEP2019
4. TITLE AND SUBTITLE **Biofidelic Three-Dimensional Brain Surrogate Models of mTBI-Induced Alzheimer's Disease Pathology**		**5a. CONTRACT NUMBER** **5b. GRANT NUMBER** W81XWH-15-1-0576 **5c. PROGRAM ELEMENT NUMBER**
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) 1. Stanford University, 3155 Porter Drive, Palo Alto, CA, 94304 2. University of Missouri, 1 Hospital Drive, Columbia, MO 65212		**8. PERFORMING ORGANIZATION REPORT NUMBER**
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012		**10. SPONSOR/MONITOR'S ACRONYM(S)** USAMRMC **11. SPONSOR/MONITOR' NUMBER(S)**

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
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

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	27	

Standard Form 298 (Rev. 8-98) Prescribed by ANSI Std. Z39.18

1. 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, cancer), neuroscience still lacks an in vitro setting to examine cellular function in 3D in densely wired, heterogeneous tissues. Novel approaches and technologies are needed that complement and advance the state-of-the art in vivo, ex vivo, and in vitro approaches to study brain physiology and diseases. Here, we are proposing 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. 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.

2. KEYWORDS:

3-D Brain Models, Alzheimer's Disease, Open-Field Blast, Traumatic Brian Injury, Neural Tissues

3. 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 level 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

- Animal protocols for isolating neurons at the Stanford University are approved by DoD.
- 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 (**Figure 1**).

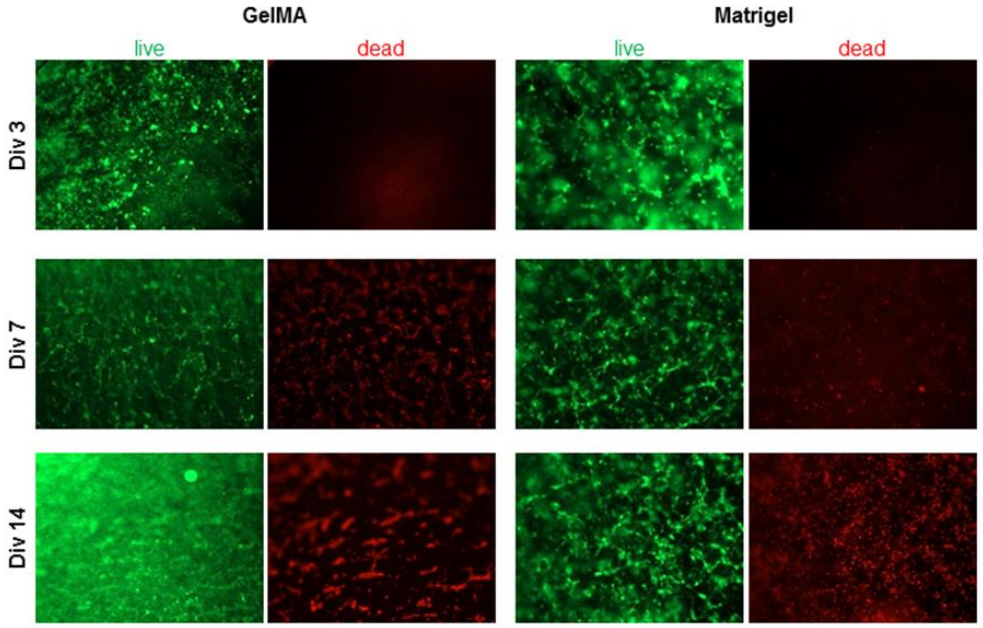

Figure 1: Comparison of viability of neurons grown in GelMA and Matrigel hydrogels. Mature neurons grown in GelMA shown higher cell viability compared to Matrigel.

- 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. They further quantified the length of functional axons, dendrites and the number of synaptic vesicles in this network (Figure 2).

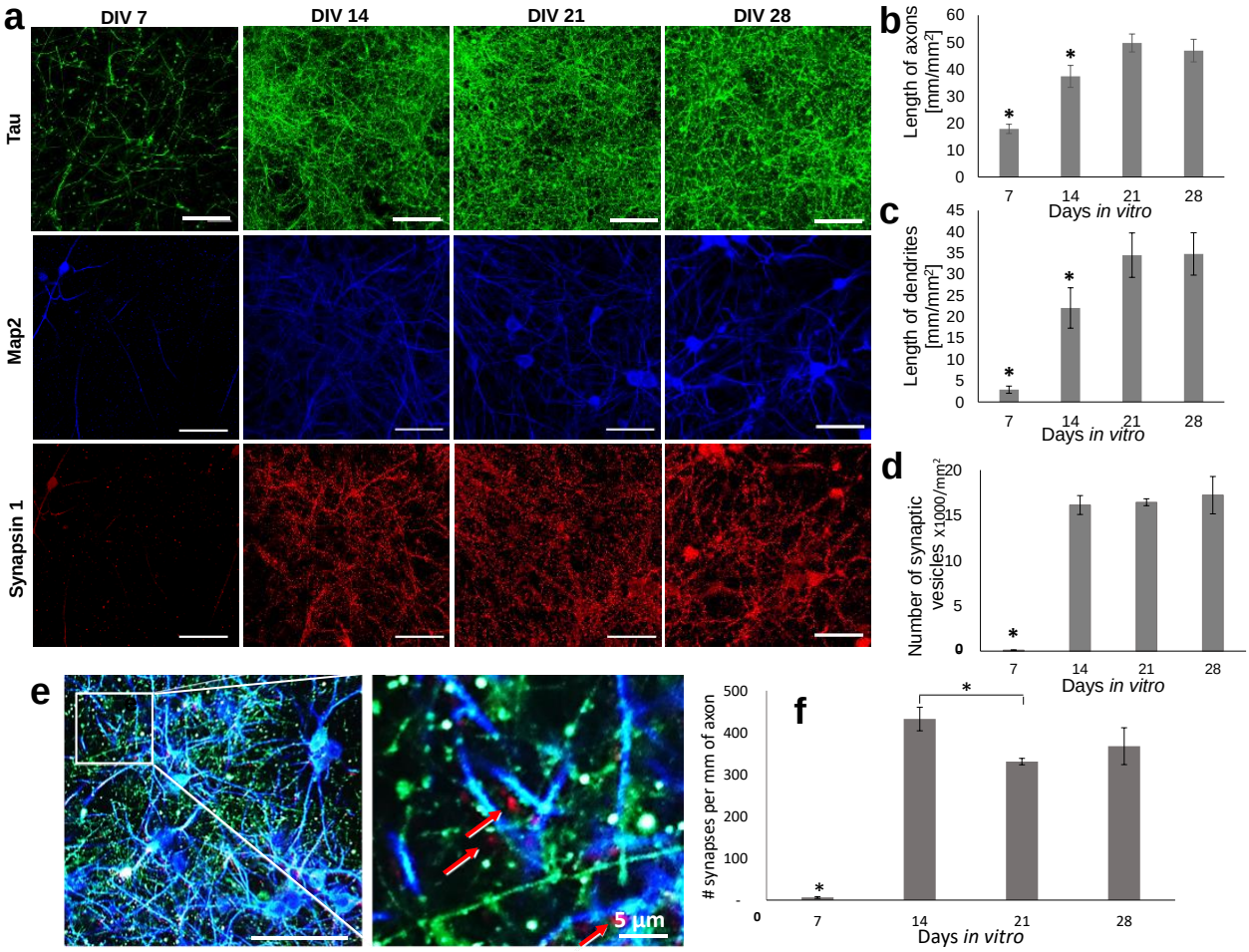

Figure 2 (a) Axo-dendritic-synapto network development of neuronal cell cultures in 3-D GelMA milli-scale block-hydrogel. Scale bars indicate 50 µm if not specifically labeled. Quantification of **(b)** axon length, **(c)** dendrite length and **(d)** synaptic vesicle number of neuronal tissues in 3-D GelMA hydrogels on day in vitro 7, 14, 21, and 28. **(e)** 2-D projection of a 3-D network formation with axons, dendrites and synapses. "Green" indicates anti-Tau staining, "blue" indicates anti-Map2 staining and "red" represents anti-synapsin-1 staining. Synapses were pointed out in the magnified image by red arrows. **(f)** Number of synaptic vesicles per mm in axon length. Standard deviation was calculated for n = 3 with a significance of $p < 0.05$ according to one way ANOVA with a Tukey post-hoc method,indicating significance with "*".

- To study neuronal network activity and their reaction to stimuli, three-week-old 3D neuronal tissues were treated with a non-competitive protein-conjugated GABAA 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 3a), and the spiking frequency and correlation coefficient also increased, however, there was no significant difference before and after PTx treatment (Figure 3b,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 3a). The pulsating curves (Figure 3d) were

further turned into a heatmap with intensity varied over time for easier comparison of frequency spikes (Figure 3e). 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 GelMA hydrogels over the course of three weeks.

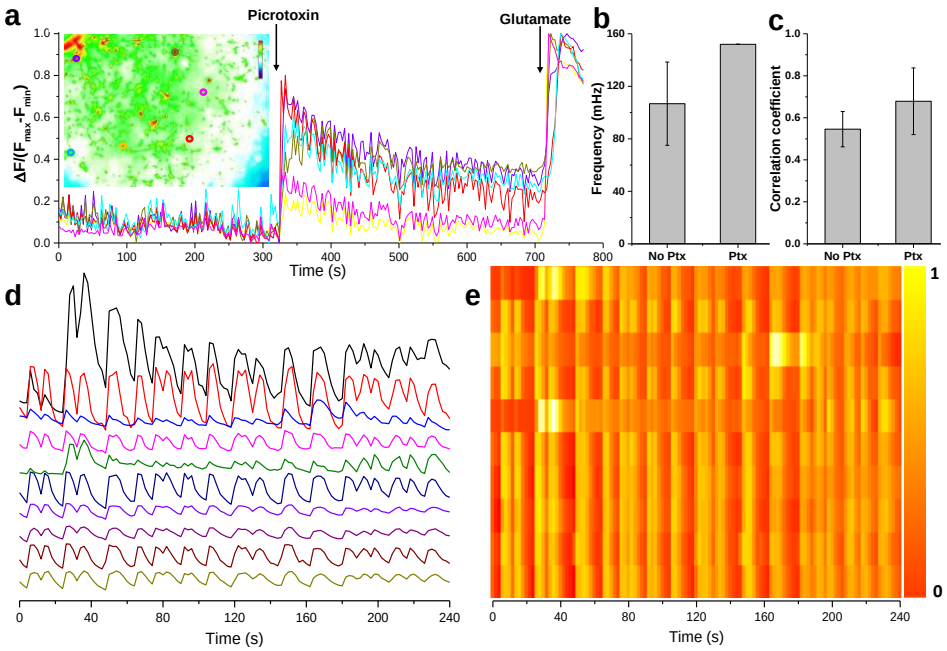

Figure 3 (a) Ca2+ intensity traces of 5 neurons marked in the image at the top left of the graph based on the video S2. Picrotoxin and Glutamate was added at 330 s and 720 s, respectively. Calcium Oscillation frequency and correlation coefficient were analysis and showed in (b). More Ca2+ (c) traces (ΔF/F0) and (d) time-series heat map of (ΔF/(Fmax-Fmin)) calculated from 10 individual neurons in milli-block which were recorded in Video S3 and selected in Figure S4. 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.

- To study neural stem cell differentiation on hydrogel surface with different stiffness. We used 3 different dilution of matrigel (pure, 1:1 diluted with culture medium and 1:2 diluted with culture medium). The pure matrigel has the highest stiffness and 1:2 has the lowest stiffness. We studied the differentiation of neural stem cell about with layer they are going to choose. The pure matrigel has the highest layer VI neurons and the 1:2 has the lowest, indicating higher modulus can increase the trend toward layer VI neurons (Figure 4).

Figure 4. Different storage moduli lead to dramatic changes in neural stem cell growth and differentiation. (a) beta-tubulin-III antibody (Tuj1, green) staining represent neuron; T-box, brain, 1 (Tbr1, red) is a transcription factor protein important in vertebrate embryo development, it's the marker of layer VI of brain cortical neuron. Nucleus were stained with DAPI (blue). (b) Statistical data show the highest surface modulus induce highest percentage of Layer VI neurons.

- Shipping condition for 3D culture neurons were tested in Demirci Lab, for DIV3, keeping at 37 °C for 24 hrs without CO2 supply can maintain 70% cell viability but for DIV7 neurons, a large percentage of cells were dead after 24 hrs.
- Animal protocols for the work at the University of Missouri (UM) are approved by DoD.
- Dr. Gu and his UM team in Columbia have worked closely with Dr. Johnson's blast team in Rolla designed, built animal holding racks and set up the blast platform holding mice on the prone position. The setting includes monitoring and measuring devices such as ultra-speed cameras and pressure gauges and connection cables, as well as detonation control and data collections.
- Animals were tested on the effects of transportation between Columbia and Rolla. There were no significant changes on behaviors (eating and drinking) were observed.
- At the current blast conditions, animal positions were not changed, monitored by high-speed camera during blast.
- No apparent gross pathological damage was found in muscles, liver, kidney, lungs. A thorough histopathological examination with hematoxylin and eosin staining including brain, lungs and liver in 7 and 30 days post blast revealed no visible pathological damage.
- We did tunnel assay and immunostaining of Iba1, GFAP, NeuN/MAP, and APP. There are no significant different on blast animal compare to sham group.
- Mouse brain tissues after blast and sham control group have been prepared well for the study on electronic microscope. The study will help us to understand the ultrastructure changes due to blast. Some results have been published (detail in publications).

- 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 (Figure 5); 3) Some mitochondria are damaged (Figure 6).
- 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.
- 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 7). 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.
- 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 8).
- 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.
- 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.
- Dr. Demirci's team has performed experiments to demonstrate the ability to grow primary neurons isolated from mouse in 3D *in vitro* cultures using different biomaterials such as Matrigel 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.

- Transportation conditions for 3D cultures between Stanford and MU were tested. The cells were not healthy after shipping with a portable incubator to MU. All cells were died.
- Dr. Gu and his team were able to perform primary neuronal cultures and have replicated the 3-D neuronal cultures at the University of Missouri-Columbia. 2D cultures were used as positive control for monitoring cell quality in Missouri.
- Missouri team has worked closely with Stanford group to test 3D cultures in Missouri, modifying the protocol and looking for the optimal culture conditions.
- Transferring 3D cultured cells between Columbia (culture location) and Rolla (blast location) would not effort the culture condition.
- To study neural stem cell differentiation, we have tried two types of 3-D culture conditions (Gelma and fibrinogen) in Missouri. We have validated the primary 3D culture conditions using fibrinogen (Figure 9) and used 2D cultures as control for monitoring cell quality in order to prepare these cultures for blast experiments.
- Tested blast conditions on 3D cultures.
- Cells were died after expose to C4 350 g (similar condition as on mice). 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.

- The intensity was reduced again (C4 was 20 g). The peak pressure at 0.45 psi induced cell death in one day after blast.
- Based on the previous data, we continue using fibrinogen 3D cultures to study neural stem cell differentiation.
- 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. We further tested conditions of open-field blast on cultured neurons, that blast cap with 0.5 grams of PETN explosive at 5.2 meters away generated peak overpressure at 0.321 PSI (2.213 kPa), rise time as 0.124 ms, and shock velocity at 366.97 m/s, which is higher than the speed of sound of 343 m/s.
- We then carried out immunofluorescent staining of neuronal markers NeuN/MAP-2 at 3 and 24 hrs post-blast. We found that in 3D-cultured cortical neurons 3 hrs after blast exposure some neuronal dendrites were observed, while 24 hrs post-blast majority of neuronal dendrites were lost and neuronal cell death. In comparison, the sham control 3D cultured neurons were with long dendritic processes and NeuN/MAP-2 positive cells (Figure 10).

- Further in vivo on mice after blast exposure revealed degenerating neuron cell bodies and dendrites using TEM analysis (Figure 11).

  Example of EM images (Figure 5):

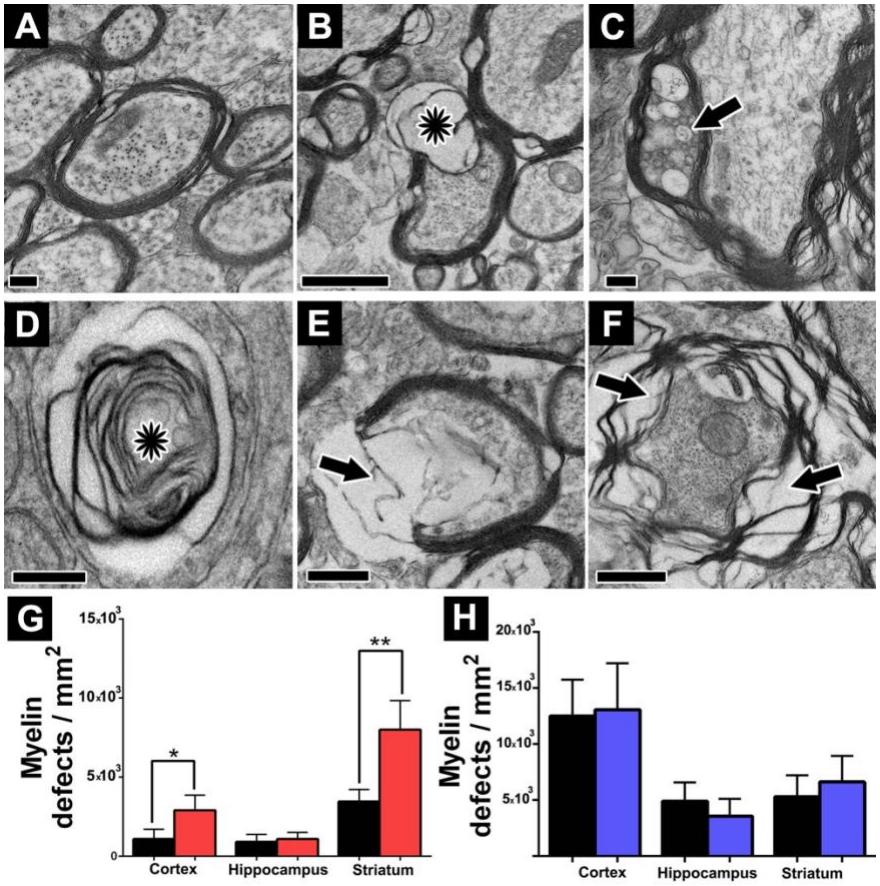

Figure 5: Myelin sheath defects identified in different brain regions after open field blast mild TBI. (A) Representative image of a normal myelinated sheath which appear as a thick, electron-dense and tightly wrapped around the axon in a sham mouse cortex. (B) “Myelin balloon” is characterized by bulges 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 asteris. (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 μm (A, C, E); 1 μm (B, D, F).

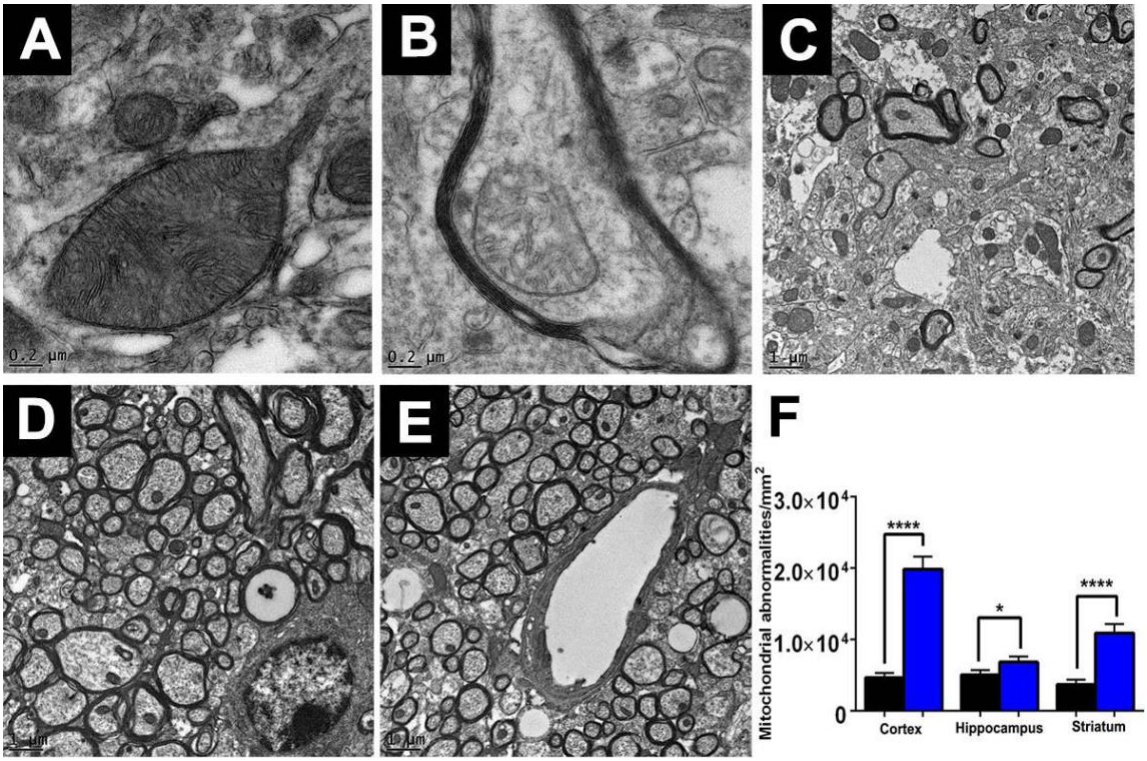

Figure 6. Abnormal ultrastructural features 30 days after blast exposure. (A and B) Mitochondria from murine cortex at high magnification, Scale bar, 0.2 μm; 10 000x magnification. (A) Normal mitochondria in a sham control cortex has a dense matrix with organized cristae; (B) Damaged mitochondrion in a myelinated axon. Notice the double membrane rupture and the swollen disorganized internal cristae. (C, D and E) Features of cytoskeletal disruption and vacuolization within the neuropil in the cortex and CC respectively. (C) Disappearance of the microtubules within the astrocytic process and dendritic process leading to an aspect of clear cytoplasm, so called “cytoplasmic aeration”. (D) Swollen myelinated axon showing a clear cytoplasm with central aggregation of microtubule. (E) Big vacuole compressing the remaining cytoplasm in myelinated axons (scale bar, 1 µm; 2000x magnification). (F) Quantification of degenerated mitochondria, Sham (black) vs Blast (blue). The blast group exhibits more degenerated mitochondria than the sham group. Student t-test; ***, $p<0.001$; ****, $p<0.0001$; $n=4$ at > 30 images per brain region.

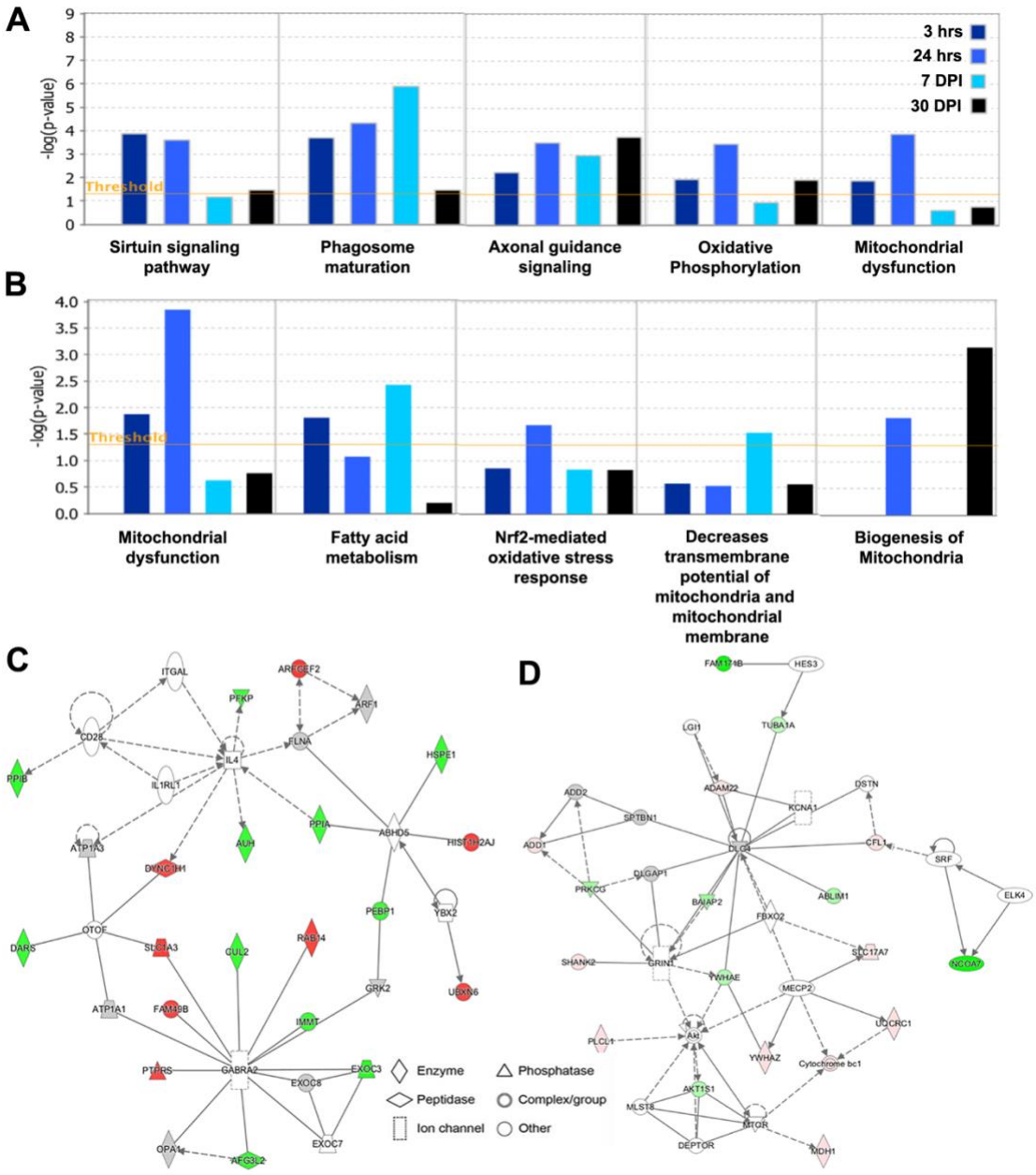

Figure 7. LIB effects at different time on alterations of canonical pathways, toxic annotation, and disease/function networks. (A) Top five canonical pathways predicted by an IPA analysis of the differentially expressed global- and phospho-proteins affected by the low-intensity blast exposure at different 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).

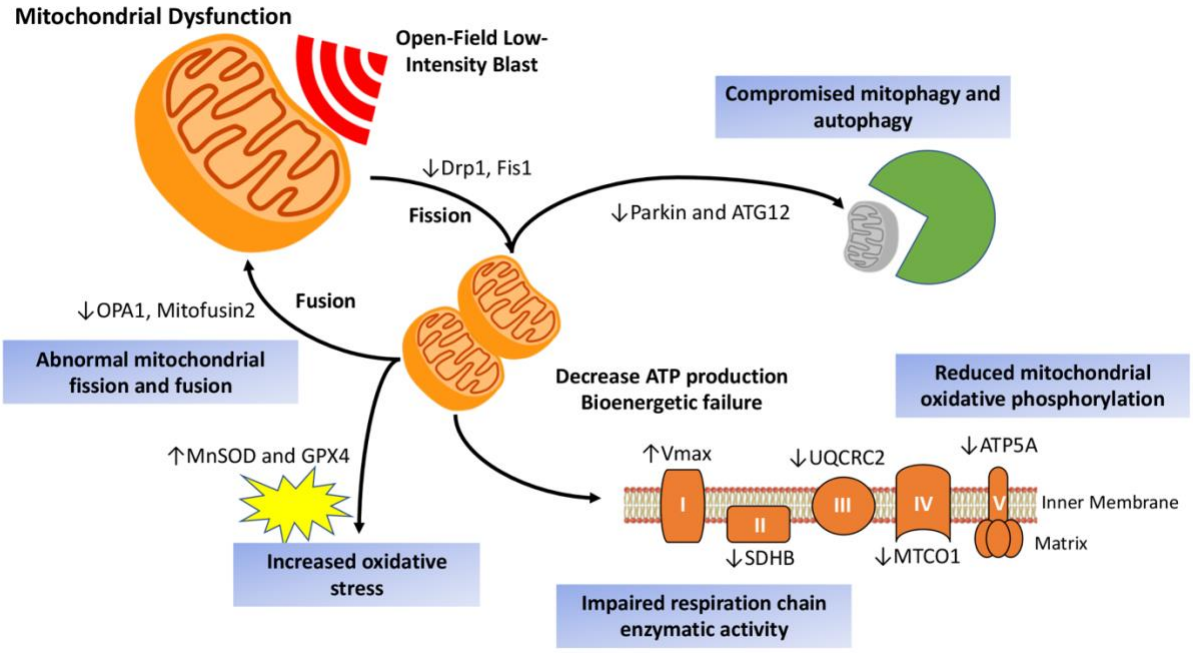

Figure 8. A scheme depicting effects of open-field low-intensity primary blast on mitochondrial dysfunction and canonical pathways. Quantitative proteomics and biochemical analysis reveal the mitochondrial dysfunction following low-intensity primary blast. We identified blast-induced mitochondrial damages associated with impaired fission-fusion dynamics, diminished mitophagy, increased oxidative stress, decreased oxidative phosphorylation, and compensated respiration activity.

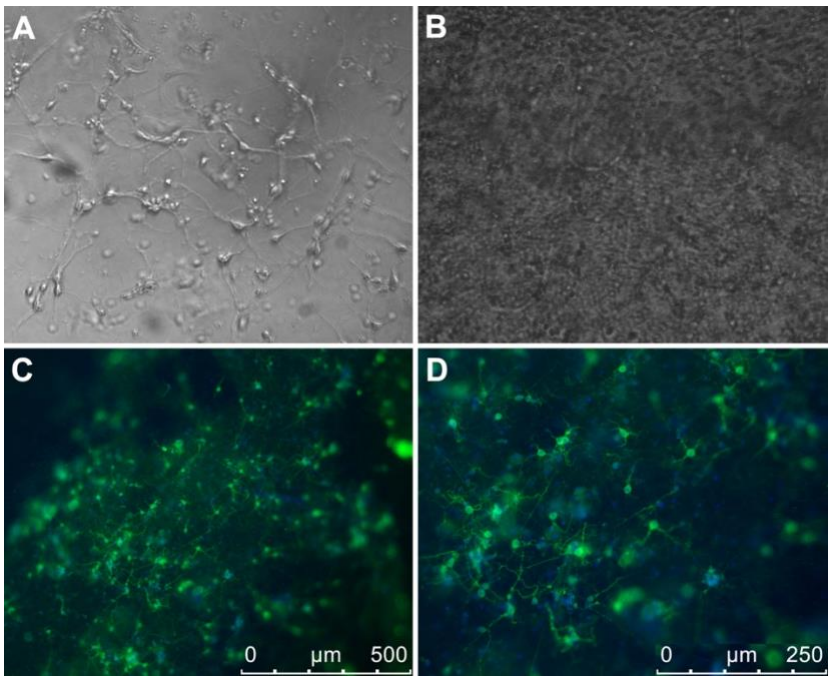

Figure 9. Conditions of neuronal cultures adopted at the University of Missouri-Columbia. (A) Representative phase contrast image of the 2D culture reveals abundant neuronal dendrites and somas, as the positive controls for the primary neuronal cultures. (B) Representative image of 3D cortical neuronal cultures before blast exposure. Some neuronal dendrites can be observed in various image focal points. (C and D) Immunofluorescent staining of neuronal markers NeuN/MAP-2 (green) with DAPI counterstaining (blue) of cell nuclei at low (C) and intermediate (D) magnifications, as the scale bars indicated.

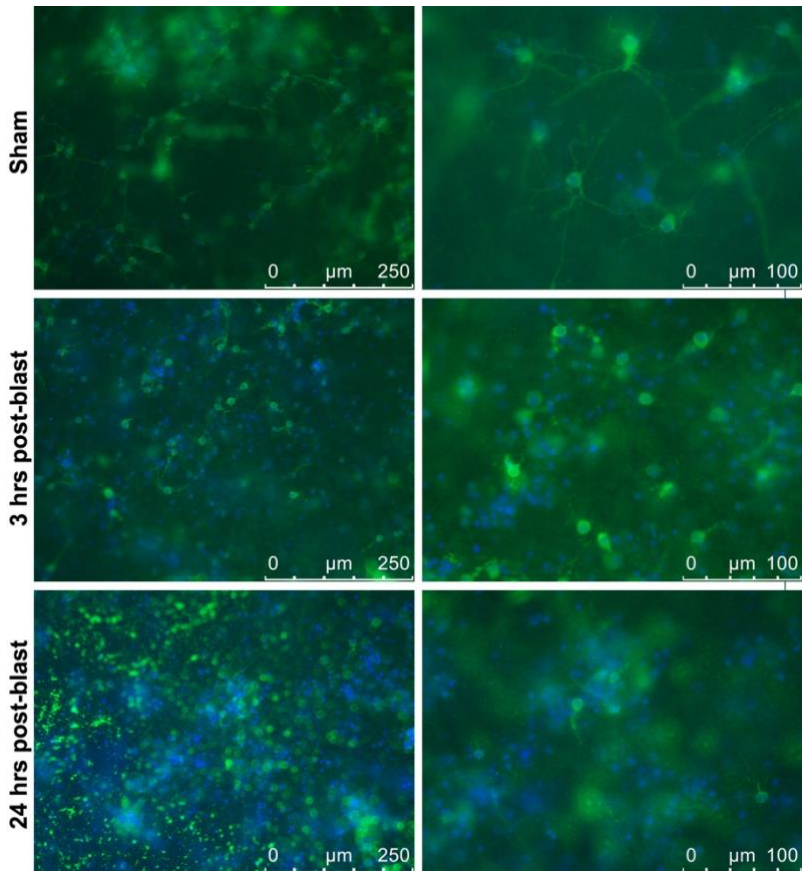

Figure 10. Immunofluorescent staining (IFS) of neuronal markers NeuN/MAP-2 (green) with DAPI counterstaining (blue) of cell nuclei for mouse cortical neurons in 3D hydrogen tissues after open-field blast exposure. (Top) Representative IFS image of the 3D culture in sham control condition reveals abundant neuronal somas and long dendrites, as the positive controls for the primary neuronal cultures. (Middle) Representative image of 3D cortical neuronal cultures 3 hrs after blast exposure. Some neuronal dendrites can still be observed. (Bottom) Representative image of 3D cortical neuronal cultures 24 hrs after blast exposure, indicating majority of dendrites are lost and neuronal cell death. Images on left column are intermediate magnification and high magnifications on right, as the scale bars indicated.

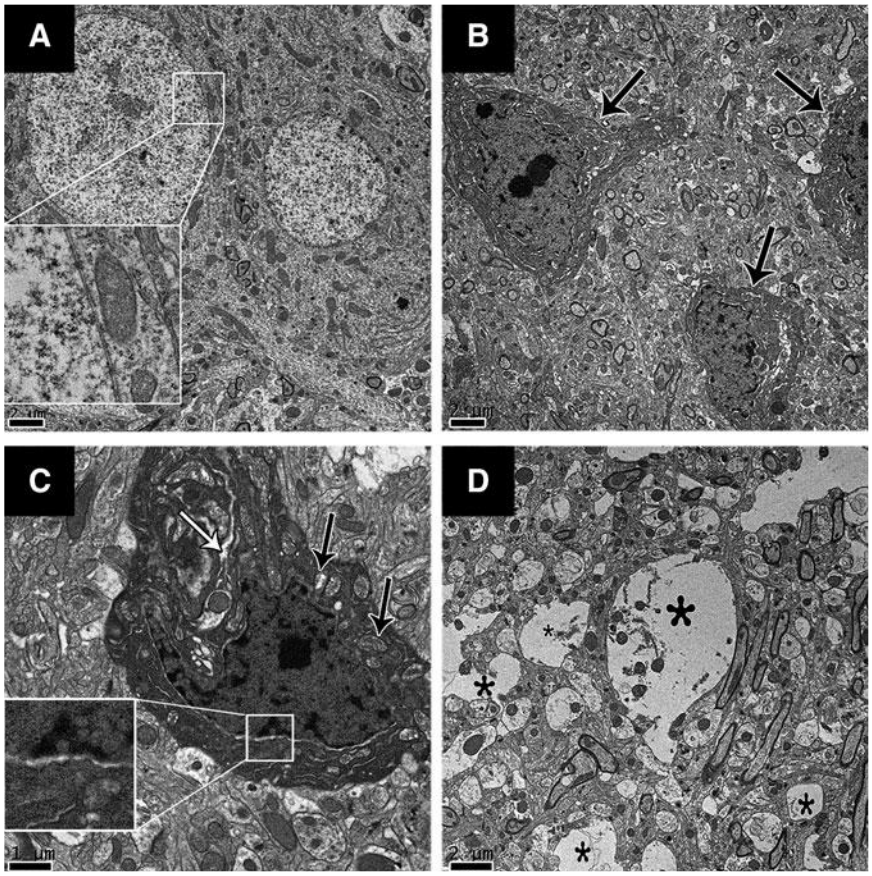

Figure 11. Neuronal cell body and dendritic abnormalities after low-intensity blast exposure. (A) Representative transmission electron microscopy images of normal pyramidal neurons in the mouse cortex in the sham control (scale = 2 μm, 800x magnification). Inset: normal mitochondria with a dense matrix containing organized, deep-protruded cristae; also the normal perinuclear membrane appears regular with close cytoplasmic contact. (B,C) Degenerating neuron cell bodies appeared dark (black arrow in B) with swollen mitochondria (black arrow in C), distended perinuclear space (inset), and distorted endoplasmic reticulum (white arrow) (B: scale = 2 μlm, 800x magnification; C: scale = 1 μm, 2000x magnification, respectively). (D) The dendritic processes after blast were swollen with a near total cytoskeletal disappearance, indicated by the asterisk (scale = 2 μm, 800x magnification).

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

In parallel with the specific aims of this project, at Stanford University, we trained and mentored several Postdoctoral researchers, medical students, graduate students, undergraduate students from Stanford and other Universities, and high school students. At University of Missouri, we trained and mentored several students such as: a) students from high schools and; b) undergraduate student from the University; and c) medical students. Students were trained with cell cultures, tissue collection, and histological analysis techniques. Under the support of the project, student from University of Missouri were able to be trained by Postdoctoral researchers from Stanford University for 3-D neuronal network building.

How were the results disseminated to communities of interest?
Results were disseminated through the following talks or presentations:

Meetings:
Stanford University
1. 2017 PRARP-CSRA Annual Review Progress Report, CDMRP Fort Detrick, MD, Feb 17, 2017
Type: oral presentation by Dr. Tanchen Ren
Title: Biofidelic 3-Dimensional Brain Surrogate Models Of mTBI Induced Alzheimer's Disease Pathology

University of Missouri
1. 2017 PRARP-CSRA Annual Review Progress Report, CDMRP Fort Detrick, MD, Feb 17, 2017
Type: oral presentation by Dr Zezong Gu
Title: Biofidelic 3-Dimensional Brain Surrogate Models Of mTBI Induced Alzheimer's Disease Pathology

2. VA GRECC Advancing Alzheimer and Aging Research: A Field Based Meeting to Foster Collaborative Multi-site Studies, Boston, MA, June 15-16, 2017
Type: oral presentation by Dr Zezong Gu
Title: S-nitrosylation and Mitochondrial Injury: A Link from Mild Blast TBI to Alzheimer

3. 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.

4. 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.

5. 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

6. 2018 The 7thWorld Chinese Mass Spectrometry Conference (WCMSC):
Type: oral presentation by Dr Zezong Gu
Title: Axonal injury and mitochondrial dysfunction associated with global and phosphorylated proteomic changes following low-intensity blast exposure

7. 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

8. 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

9. 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

10. 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

11. 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?

12. DOD Working Group on Computational Modeling of Human Lethality, Injury, and Impairment from Blast-related Threats on Feb 5-6, 2019 at MITRE #4, Mclean, VA
Type: oral presentation by Dr Zezong Gu
Title: Multifocal Ultrastructural Abnormalities in Neurons, Axons and Synapses after Low-Intensity Blast (LIB) Exposure: Scaling and Computational Challenges

13. VA/VHA/VACO Site Visit to Truman VAMC/UM on Mar 22, 2019
Type: oral presentation by Dr Zezong Gu
Title: Primary blast-induced mild TBI linked to cognitive dysfunctions in neurodegenerative disorders

14. 2019 State of the Science Summit Paths to Treatment for Traumatic Brain Injury(s) Jun 5-6, 2019 at Washington DC
Type: Working group discussions for PreClinical/Translational Domain on Day 1 for Review of synthesis/identification of gaps and new directions; and on Day 2 to Identify & prioritize opportunities to address key gaps & new directions.
Participants: Fiona Crawford, Stephen Ahler, Patrick Kochanek, Susanna Rosi, Doug Smith, Ina Wanner, Jiankun Cui, Zezong Gu, etc.; Moderator: Chantelle Ferland-Beckham; Closing Session remarks by VA CRADO Rachel Ramoni highlighted the "Missouri Blast" as a nation-wide resource for preclinical studies.

15. NeuroTrauma 2019 in Pittsburgh at Jun 30 to Jul 3, 2019
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

What do you plan to do to accomplish the goals?

We aim to seek new DoD support and continue into the next phase of our studies.

4. IMPACT:
What was the impact on the development of the principal discipline(s) of the project?

This bioengineering approach presented several crucial advances and benefits for studying mTBI/AD. Here, we first provide 3D model systems for developing the most naturalistic approximation of the brain *in vitro,* thus elucidating the etiology of human brain disorders. We also examine the chemical composition of hydrogels for dense encapsulation of neurons in 3D geometries for generating a more appropriate microenvironment than the aqueous nature of conventional 2D cultures. We provide specific cell manipulation techniques in 3D systems, which are the same ease of manipulation as traditional 2D neuronal cultures in neuroscience research, as well as minimize challenges *in vivo* systems, including cost, limitation in analysis, throughput and freedom of manipulation, risk of inconsistent readout due to animal variability and need of skilled technical personnel. By combining these multiple aspects, we demonstrate an excellent mimicry model to study the impact of brain trauma in general, and in particular of shock waves that cause ultrastructural damage and loss of brain functionality of as it relates to mTBI/Alzheimer's disease pathology.
What was the impact on other disciplines?

In addition to the neurobiology, the presented bioengineering approach has broad impact on multiple disciplines, including genetics, molecular biology, biomaterials, biophysics, chemistry, biomimetics, and bioinspired technologies.

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

This bioengineering approach has great impact over civil and army personnel having mild traumatic brain injury or Alzheimer's disease (AD) associated pathology. This approach will open new avenues to understand the mechanism(s) that cause blast induced mTBI/AD pathology, and it will especially help the diagnosis, monitoring, prevention and treatment of brain damage in soldiers. Compared to existing *in vivo* and *ex vivo* systems, biofidelic 3D systems are easy-to-use, cost-effective, and allow multiple parallel testing of many experimental conditions.

From a broad perspective, this approach provides a versatile platform that is potentially adapted to other neurologic disorders (e.g., Parkinson's disease), infection diseases, and noncommunicable diseases such as diabetes and cancer.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Tissue cultures and blast conditions were changed. At similar blast conditions on mice, all of cells died in 24 hrs.

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

Cell death still happened even when we reduced the blast conditions from 6.7 psi (similar condition on mice: C4 350g, 3 m distance of blast) to 0.46 psi (C4 20g, 9 m distance from blast). We are seeking alternate ways to reduce the blast impact on cell, either reduce the blast psi or putting cell in the water tank, for example, as literatures reported.

Further tests indicated the blast conditions on 3D cultures significantly different than the in vivo rodent's blast given the lack of a skull to protect the neurons. We further tested conditions of open-field blast, using Blast cap (a small sensitive primary blast device) with 0.5 grams of PETN explosive at 5.2 meters away generated peak overpressure at 0.321 PSI (2.213 kPa), rise time as 0.124 ms, and shock velocity at 366.97 m/s, which is higher than the speed of sound of 343 m/s. Under such blast conditions, 3D-cultured cortical neurons revealed mild to moderate neurodegeneration examined by IFS with neuronal markers NeuN/MAP-2 showing different degrees in loss of neuronal dendrites and cell death, similar findings of neuronal dendritic degeneration observed in the in vivo rodent blast studies.

Changes that had a significant impact on expenditures

Nothing to report.

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

Nothing to report.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals.

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:
Publications.
Journal publications.

1. Hailong Song, Jiankun Cui, Agnes Simonyi, Catherine E. Johnson, Graham K. Hubler, Ralph G. DePalma, and Zezong Gu. Linking Blast Physics to Biological Outcomes in Mild Traumatic Brain Injury: Narrative Review and Preliminary Report of an Open-Field Blast Model. ***Behavioural Brain Research*** 2018 Mar 15;340:147-158. doi: 10.1016/j.bbr.2016.08.037. Epub 2016 Aug 21.

2. 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)

3. 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.

4. 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

5. 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

6. Konan LM*, Song H*, Pentecost G, Fogwe D, Ndam T, Cui J, Johnson CE, Grant D, White T, Chen M, Xia W, Cernak I, DePalma RG, Gu Z. Multi-Focal Neuronal Ultrastructural Abnormalities and Synaptic Alterations in Mice after Low-Intensity Blast Exposure. J Neurotrauma. 2019 Jul 1;36(13):2117-2128. doi: 10.1089/neu.2018.6260.

7. 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;181:402-14

8. Ren, T., Grosshäuser, B., Sridhar, K., Nieland, J.F.T., Tocchio, A., Schepers, U., **Demirci, U.**, "3-D geometry and irregular

connectivity dictate neuronal firing in frequency domain and synchronization" Biomaterials, 2019, 197, 171-181

9. "Packing neuronal cells in Buckyballs," Tanchen Ren, Wolfgang Steiger, Pu Chen[3,4], Mehmet Giray Ogut, Aleksandr Ovsianikov*, Utkan Demirci* (Under review)

10. "Soft ring-shaped neuronal cellu-robots with simultaneous locomotion in batches," Tanchen Ren, Pu Chen, Longjun Gu, Utkan Demirci (Under review)

Books or other non-periodical, one-time publications

Nothing to report.

Other publications, conference papers, and presentations.

1. 2017 PRARP-CSRA Annual Review Progress Report, CDMRP Fort Detrick, MD, Feb 17, 2017
Type: oral presentation by Dr Zezong Gu
Title: Biofidelic 3-Dimensional Brain Surrogate Models Of mTBI Induced Alzheimer's Disease Pathology

2. VA GRECC Advancing Alzheimer and Aging Research: A Field Based Meeting to Foster Collaborative Multi-site Studies, Boston, MA, June 15-16, 2017
Type: oral presentation by Dr Zezong Gu
Title: S-nitrosylation and Mitochondrial Injury: A Link from Mild Blast TBI to Alzheimer

3. 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.

4. 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.

5. 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

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

12. Canadas, R., Ren, T., Marques, A.P., Oliveira, J.M., Reis, R.L., Demirci, U., “Biochemical Gradients to Form a 3-D Osteochondral in Vitro Model.” TERMIS European Chapter Meeting, Rhodes, Greece, 2019 (oral presentation)

13. Guest Lecture “3-D bioprinting technologies and tools”, in the Advances in Biotechnology Class (BioE450), Department of Bioengineering, Stanford University

14. Chemistry-Free Microfluidic Technologies to Sort Cells for Health and Disease/Invited Talk, Circulating Biomarkers World Congress, San Diego, CA

15. Chemistry-free Micro-fluidic Technologies to Sort Cells for Health and Disease / Research Presentation, Translational Science in Musculoskeletal Disorders Workshop, Guimarães, Portugal (Invited Talk)

16. Assembling 3D Tissue Constructs Using Label-free Magnetic Additive Biomanufacturing Technologies/Research presentation, University of Toronto MIE Distinguished Seminar Series, Toronto, Canada (Invited Talk)

Website(s) or other Internet site(s)

Nothing to report.

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Nothing to report.

Other Products

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Utkan Demirci
Project Role: PI
Research Identifier: utkandemirci (NIH agency login)
Month worked: 0month
Contribution to Project: Supervision of project at Stanford
Name: Zezong Gu
Project Role: co-PI
Research Identifier: zegunih1 (NIH agency login)
Month worked: 0.6 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, experimental design and performance, analyzing data, writing reports and manuscript.

Nearest person: Jiankun Cui
Project Role: Research Assistant Professor and Senior Personnel
Research Identifier: none
Month worked: 0.98 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: 1 months
Contribution to Project:
In year 4, he had been involved revising manuscript, seeking alternative methods for the blast condition on cells.

Name: Runting Li
Project Role: Lab technician
Research Identifier: none
Month worked: 0.5 months
Contribution to Project:
General lab work and assistant PI and others in Gu lab for the project, involving, but not limited, order supplies, and assistant some blast experiments, cell culture, immunostaining.

Person: Catherine E Johnson and her team
Project Role: Engineer
Research Identifier: none
Month worked: 8 hrs
Contribution to Project:
Dr. Johnson worked closed with Dr. Gu and his team on blast experimental design, blast settings.
Under Dr.Johnson's supervision, the team has performed the blast in Rolla and analyzed physical data from blast sitting.

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?

Organization Name: University of Missouri
Location of Organization: Columbia, MO
Partner's contribution to the project

Collaboration: The University of Missouri is a subcontract on this grant. Their personnel and contributions are listed above.

8. **SPECIAL REPORTING REQUIREMENTS**
    a. **COLLABORATIVE AWARDS:**
        Nothing to report.
    b. **QUAD CHARTS:**
        Nothing to report.
9. **APPENDICES:**
    a. Publication