REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
25-01-2019	Final Report	22-Jul-2013 - 21-Oct-2014

4. TITLE AND SUBTITLE

Final Report: Biaxial Test System for Biomechanical Testing in Trauma Research and Bioengineering Education

5a. CONTRACT NUMBER
W911NF-13-1-0260

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611103

6. AUTHORS

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES

University of Pennsylvania
Office of Research Services
3451 Walnut Street, Suite P-221
Philadelphia, PA 19104 -6205

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES)

U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
63330-LS-RIP.7

12. DISTRIBUTION AVAILIBILITY STATEMENT

Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Beth Winkelstein
UU	UU	UU	UU		19b. TELEPHONE NUMBER 215-573-4589

RPPR Final Report
as of 30-Jan-2019

Agency Code:

Proposal Number: 63330LSRIP **Agreement Number: W911NF-13-1-0260**
INVESTIGATOR(S):

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Country: USA
DUNS Number: 042250712 EIN: 231352685

Report Date: 21-Jan-2015 Date Received: 25-Jan-2019
Final Report for Period Beginning 22-Jul-2013 and Ending 21-Oct-2014
Title: Biaxial Test System for Biomechanical Testing in Trauma Research and Bioengineering Education
Begin Performance Period: 22-Jul-2013 **End Performance Period:** 21-Oct-2014
Report Term: 0-Other

Submitted By: Beth Winkelstein Email: winkelst@seas.upenn.edu
Phone: (215) 573-4589

STEM Degrees: 5 **STEM Participants:** 2

Major Goals: This was an equipment grant to acquire the BioTester Biaxial Test System for mechanical testing of biologic tissues and biomaterials to enable experiments to define and study trauma, injury, pain and novel biomaterials to promote wound healing and relieve pain. Understanding the mechanotransduction mechanisms that drive pain and cellular dysfunction brought about by mechanical tissue loading is a key element contribute to significantly enhancing the prevention and treatment of injury. The test system was essential for advancing studies by providing appropriate loading and data acquisition at a physical scale not otherwise available. In addition to advance experiments and benefiting computational models, it also provided valuable experiences for trainees at the undergraduate and graduate levels introducing them to state-of-the-art techniques and analysis of biomechanics and biomaterials applications. The requested instrumentation catalyzed several existing synergistic research activities in trauma-related injuries and significantly enhanced the research-related education in Bioengineering and Physiology at Penn.

Accomplishments: This equipment grant facilitated and substantially enhanced several projects defining the mechanotransduction of trauma-induced pain. During the award period alone, many publications were produced (see other section), as well as undergraduate and graduate students trained in STEM (see other section) with this biaxial test system. The results of experiments from this test system have defined thresholds at the cellular level for the first time that relate pain, dysfunction and injury.

This was an equipment grant so the most important result is acquiring and installing the equipment that then was used to perform several experiments. Among those are the finding that neural activation is induced at the same strain threshold as that when its surrounding collagen matrix becomes disorganized.

RPPR Final Report
as of 30-Jan-2019

Training Opportunities: Number of Undergraduate STEM Students:

Since this is an equipment grant, no undergraduate students were financially supported from these funds. However, 2 undergraduate students were involved in at least one study that was submitted during the award period.

Number of Graduate STEM Students:

Since this is an equipment grant, no personnel were supported from these funds. However, Sijia Zhang (graduate students in Bioengineering) used the equipment extensively in experiments as part of her PhD thesis. Dr. Zhang defended her PhD thesis in Fall 2017 and went on to a post-doctoral position at UCSF. In addition, through ongoing collaboration, Vahhab Zarei (graduate student in Biomedical Engineering at University of Minnesota) used much of Dr. Zhang's data in his computational modeling PhD these. Lastly, Jenell Smith, Samantha Schumm and Meagan Ita (graduate students in Bioengineering) also participated in studies that benefitted from this equipment. Of note, Meagan Ita continues to work with this equipment on a project that is central to her PhD thesis.

Number of students that received a STEM degree:

Because of its being an equipment grant, no students were financially supported by this award. However, 5 PhD students used this equipment directly or indirectly in studies as part of their doctoral dissertations, and an additional 2 undergraduates were involved in those studies and/or additional experiments which benefited from the system.

Other Research Staff:

This equipment grant did not cover personnel support. However, the testing system has been used by 2 additional faculty in the School of Engineering and Applied Science and has been used in our collaboration with colleagues at University of Minnesota, being used to support the research activities within the Barocas Lab in Biomedical Engineering. Dr. Robert Carpick (Mechanical Engineering and Applied Mechanics) and Dr. Kacy Cullen (Neurosurgery & Bioengineering) have used the biaxial test set-up and imaging for pilot studies during the project period.

RPPR Final Report
as of 30-Jan-2019

Results Dissemination: Papers in Peer-Reviewed Journals

We submitted 6 full-length manuscripts for studies that directly used equipment from this DURIP; all were or have since been published.

Smith JR, Galie PA, Slochower DR, Weisshaar CL, Janmey PA, Winkelstein BA. Salmon-derived thrombin inhibits development of neuropathic pain through an endothelial barrier protective mechanism dependent on APC. Biomaterials, 80:96-105, 2016.

Zhang S, Cao X, Stablow AM, Shenoy VB, Winkelstein BA. Tissue strain reorganizes collagen with a switchlike response that regulates neuronal extracellular signal-regulated kinase phosphorylation in vitro: Implications for ligamentous injury and mechanotransduction. Journal of Biomechanical Engineering, 138(2):021013, 2016.

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Ban E, Zhang S, Zarei V, Barocas VH, Winkelstein BA, Picu CR. Collagen organization in facet capsular ligaments varies with spinal region and with ligament deformation. Journal of Biomechanical Engineering, 139(7):071009-1, 2017.

Ita ME, Zhang S, Holsgrove, Kartha S, Winkelstein BA. The physiological basis of cervical facet-mediated persistent pain: Basic science and clinical challenges. Journal of Orthopedic & Sports Physical Therapy, 47(7):450-461, 2017.

Zarei V, Zhang S, Winkelstein BA, Barocas VH. Tissue loading and microstructure regulate the deformation of embedded nerve fibers: Predictions from single-scale and multiscale simulations. Journal of the Royal Society Interface, 14(135):20170326, 2017.

Papers in Non-Peer-Reviewed Journals: None

Presentations:

During the award period we presented 6 abstracts and submitted another 3 that were later presented at conferences.

Zhang S, Barocas V, Winkelstein B. Local Neuronal Loading Modulates pERK Expression in a Neuron-Collagen Gel Construct Simulating Facet Capsule Injury. 7th World Congress of Biomechanics, #W229, Boston, MA, July 2014.

Zitnay JL, Lake SP, Quinn KP, Lee DJ, Winkelstein BA, Barocas V. Multiscale Modeling of the Cervical Facet Capsular Ligament During Tensile Joint Loading. 7th World Congress of Biomechanics, #111-12, Boston, MA, July 2014.

Barocas VH, Akkin T, Shephard M, Winkelstein BA. Multiscale Modeling of Facet Capsule Mechanobiology: Integrating Modeling and Experimental Techniques. Multiscale Modeling Consortium Meeting, #31, Bethesda, MD, September 2014.

Zhang S, Cao X, Shenoy VB, Winkelstein BA. Multiscale Approaches to Understanding Microscopic Collagen Fiber Mechanics Under Macroscopic Loading of Facet Capsular Ligaments. Global Conference on Nanoengineering for Medicine and Biology, #NEMB2015-8137, April 2015.

Zhang S, Cao X, Shenoy VB, Winkelstein BA. Defining Collagen Fiber Mechanics in Neuron-Collagen Constructs Under Stretch Using Integrated Experimental & Modeling Approaches. Summer Biomechanics, Bioengineering & Biotransport Conference, #SB3C2015-281, Snowbird, UT, June 2015.

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Zhang S, Winkelstein BA. The RhoA/ROCK Pathway Mediates Nociceptive Signaling after Painful Ligament Loading. Summer Biomechanics, Bioengineering & Biotransport Conference, #SB3C2016-32, National Harbor, MD, June 2016.

Schumm SN, Ita ME, Winkelstein BA. Characterization of the failure responses of skin: Comparison of uniaxial and equibiaxial planar mechanics. Summer Biomechanics, Bioengineering & Biotransport Conference, #SB3C2016-649, National Harbor, MD, June 2016.

Ita ME, Winkelstein BA. Collagen degradation alters failure properties & matrix reorganization during tensile loading. Summer Biomechanics, Bioengineering & Biotransport Conference, #SB3C2016-92, National Harbor, MD, June 2016.

Honors and Awards: The PI has been recognized in several ways due to her activities using this equipment, and related to the equipment in this proposal. During the award period, Dr. Winkelstein was elected as a Fellow of the American Institute for Medical and Biological Engineering (2013) and Biomedical Engineering Society (2014). Our work using this system has been recognized as an Editors' Choice top paper in 2016 by the Journal of Biomechanical Engineering. In addition, a graduate student Sijia Zhang who used this equipment was awarded the AMTI Force and Motion Scholarship (in 2016) for proposed work using the system. Meagan Ita (graduate student) was recognized by an NSF Fellowship Honorable Mention. Through our collaborations with computational modelers, we submitted an U01 application in 2016 which has since been awarded.

Protocol Activity Status:

Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: Graduate Student (research assistant)
Participant: Sijia Zhang
Person Months Worked: 12.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: Jenell Smith
Person Months Worked: 12.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: Samantha Schumm
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)

RPPR Final Report
as of 30-Jan-2019

Participant: meagan Ita
Person Months Worked: 12.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Undergraduate Student
Participant: Preston Eni
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Undergraduate Student
Participant: Harrison Troche
Person Months Worked: 8.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Faculty
Participant: Kacy Cullen
Person Months Worked: 2.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: PD/PI
Participant: beth Winkelstein
Person Months Worked: 15.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

RPPR Final Report
as of 30-Jan-2019

Nothing to Report in the uploaded PDF (see accomplishments and other sections)
Below summarizes information uploaded.
Statement of Problem Studied: This was an equipment grant so the goal was to build and implement a biaxial tester system to enable controlled loading of tissues and in vitro constructs to understand injury and trauma induced neuronal and collagen dysregulation.

Summary of Most Important Results: This was an equipment grant so the most important result is acquiring and installing the equipment that then was used to perform several experiments. Among those are the finding that neural activation is induced at the same strain threshold as that when its surrounding collagen matrix becomes disorganized.

Report Date: 1/20/19

Report Type & Dates Covered: Award Date: Final Report; Award Period: 7/22/13–10/21/14

Proposal Title: Biaxial Test System for Biomechanical Testing in trauma Research and Bioengineering Education

Grant Number: W911NF-13-1-0260

Author: Beth A. Winkelstein, PhD

Performing Organization Name & Address: Trustees of the University of Pennsylvania, 3451 Walnut St., Philadelphia, PA 19104; equipment resides in 415 Skirkanich Hall, 240 S 33rd St., Philadelphia PA 19104

Abstract:
This was an equipment grant to acquire the BioTester Biaxial Test System for mechanical testing of biologic tissues and biomaterials to enable experiments to define and study trauma, injury, pain and novel biomaterials to promote wound healing and relieve pain. Understanding the mechanotransduction mechanisms that drive pain and cellular dysfunction brought about by mechanical tissue loading is a key element contribute to significantly enhancing the prevention and treatment of injury. The test system was essential for advancing studies by providing appropriate loading and data acquisition at a physical scale not otherwise available. In addition to advance experiments and benefiting computational models, it also provided valuable experiences for trainees at the undergraduate and graduate levels introducing them to state-of-the-art techniques and analysis of biomechanics and biomaterials applications. The requested instrumentation catalyzed several existing synergistic research activities in trauma-related injuries and significantly enhanced the research-related education in Bioengineering and Physiology at Penn.

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phosphorylation in vitro: Implications for ligamentous injury and mechanotransduction. *Journal of Biomechanical Engineering,* 138(2):021013, 2016.

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Ban E, Zhang S, Zarei V, Barocas VH, **Winkelstein BA**, Picu CR. Collagen organization in facet capsular ligaments varies with spinal region and with ligament deformation. *Journal of Biomechanical Engineering*, 139(7):071009-1, 2017.

Ita ME, Zhang S, Holsgrove, Kartha S, **Winkelstein BA**. The physiological basis of cervical facet-mediated persistent pain: Basic science and clinical challenges. *Journal of Orthopedic & Sports Physical Therapy,* 47(7):450-461, 2017.

Zarei V, Zhang S, **Winkelstein BA**, Barocas VH. Tissue loading and microstructure regulate the deformation of embedded nerve fibers: Predictions from single-scale and multiscale simulations. *Journal of the Royal Society Interface,* 14(135):20170326, 2017.

Papers in Non-Peer-Reviewed Journals: None

Presentations:

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Schumm SN, Ita ME, Winkelstein BA. Characterization of the failure responses of skin: Comparison of uniaxial and equibiaxial planar mechanics. *Summer Biomechanics, Bioengineering & Biotransport Conference*, #SB^{3}C2016-649, National Harbor, MD, June 2016.

Ita ME, Winkelstein BA. Collagen degradation alters failure properties & matrix reorganization during tensile loading. *Summer Biomechanics, Bioengineering & Biotransport Conference*, #SB^{3}C2016-92, National Harbor, MD, June 2016.

Manuscripts: None

Books: None

Honors & Awards

The PI has been recognized in several ways due to her activities using this equipment, and related to the equipment in this proposal. During the award period, Dr. Winkelstein was elected as a Fellow of the American Institute for Medical and Biological Engineering (2013) and Biomedical Engineering Society (2014). Our work using this system has been recognized as an Editors' Choice top paper in 2016 by the *Journal of Biomechanical Engineering*. In addition, a graduate student Sijia Zhang who used this equipment was awarded the AMTI Force and Motion Scholarship (in 2016) for proposed work using the system. Meagan Ita (graduate student) was recognized by an NSF Fellowship Honorable Mention. Through our collaborations with computational modelers, we submitted an U01 application in 2016 which has since been awarded.

Patents: None.

Number of Undergraduate STEM Students:

Since this is an equipment grant, no undergraduate students were financially supported from these funds. However, 2 undergraduate students were involved in at least one study that was submitted during the award period.

Number of Graduate STEM Students:

Since this is an equipment grant, no personnel were supported from these funds. However, Sijia Zhang (graduate students in Bioengineering) used the equipment extensively in experiments as part of her PhD thesis. Dr. Zhang defended her PhD thesis in Fall 2017 and went on to a post-doctoral position at UCSF. In addition, through ongoing collaboration, Vahhab Zarei (graduate student in Biomedical Engineering at University of Minnesota) used much of Dr. Zhang's data in his computational modeling PhD these. Lastly, Jenell Smith, Samantha Schumm and Meagan Ita (graduate students in Bioengineering) also participated in studies that benefitted from this equipment. Of note, Meagan Ita continues to work with this equipment on a project that is central to her PhD thesis.

Number of students that received a STEM degree:

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Other Research Staff:

This equipment grant did not cover personnel support. However, the testing system has been used by 2 additional faculty in the School of Engineering and Applied Science and has been used in our collaboration with colleagues at University of Minnesota, being used to support the research activities within the Barocas Lab in Biomedical Engineering. Dr. Robert Carpick (Mechanical Engineering and Applied Mechanics) and Dr. Kacy Cullen (Neurosurgery &

Bioengineering) have used the biaxial test set-up and imaging for pilot studies during the project period.

Technology Transfer: None.

Scientific Progress and Accomplishments:

This equipment grant facilitated and substantially enhanced several projects defining the mechanotransduction of trauma-induced pain. During the award period alone, many publications were produced (see above), as well as undergraduate and graduate students trained in STEM (see above) with this biaxial test system. The results of experiments from this test system have defined thresholds at the cellular level for the first time that relate pain, dysfunction and injury.

Copies of Technical Reports: None.