

REPORT DOCUMENTATION PAGE			Form Approved OMB NO. 0704-0188		
The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA, 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE (DD-MM-YYYY) 25-01-2019		2. REPORT TYPE Final Report		3. DATES COVERED (From - To) 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 AVAILABILITY 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 be construed 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 UU	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON Beth Winkelstein
a. REPORT UU	b. ABSTRACT UU	c. THIS PAGE 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):

Name: Paul Janmey
Email: janmey@mail.med.upenn.edu
Phone Number: 2155736813
Principal: N

Name: Beth A. Winkelstein
Email: winkelst@seas.upenn.edu
Phone Number: 2155734589
Principal: Y

Organization: **University of Pennsylvania**

Address: Office of Research Services, Philadelphia, PA 191046205

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

Distribution Statement: 1-Approved for public release; distribution is unlimited.

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.

Sperry MM, Ita ME, Kartha S, Zhang S, Yu Y-H, Winkelstein BA. The interface of mechanics and nociception in joint pathophysiology: Insights from the facet & temporomandibular joints. *Journal of Biomechanical Engineering*, 139(2):021003-2, 2017.

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.

Smith JR, Galie PA, Slochower DR, Janmey PA, Winkelstein BA. Salmon Thrombin Inhibits Endothelial Barrier Disruption Through its Activation of Protein C. Summer Biomechanics, Bioengineering & Biotransport Conference, #SB3C2015-273, Snowbird, UT, June 2015.

RPPR Final Report as of 30-Jan-2019

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

Project Contribution:

International Collaboration:

International Travel:

National Academy Member: N

Other Collaborators:

Funding Support:

Participant Type: Undergraduate Student

Participant: Preston Eni

Person Months Worked: 3.00

Project Contribution:

International Collaboration:

International Travel:

National Academy Member: N

Other Collaborators:

Funding Support:

Participant Type: Undergraduate Student

Participant: Harrison Troche

Person Months Worked: 8.00

Project Contribution:

International Collaboration:

International Travel:

National Academy Member: N

Other Collaborators:

Funding Support:

Participant Type: Faculty

Participant: Kacy Cullen

Person Months Worked: 2.00

Project Contribution:

International Collaboration:

International Travel:

National Academy Member: N

Other Collaborators:

Funding Support:

Participant Type: PD/PI

Participant: beth Winkelstein

Person Months Worked: 15.00

Project Contribution:

International Collaboration:

International Travel:

National Academy Member: N

Other Collaborators:

Funding Support:

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.

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.

Sperry MM, Ita ME, Kartha S, Zhang S, Yu Y-H, **Winkelstein BA**. The interface of mechanics and nociception in joint pathophysiology: Insights from the facet & temporomandibular joints. *Journal of Biomechanical Engineering*, 139(2):021003-2, 2017.

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.

Smith JR, Galie PA, Slochower DR, Janmey PA, Winkelstein BA. Salmon Thrombin Inhibits Endothelial Barrier Disruption Through its Activation of Protein C. *Summer Biomechanics, Bioengineering & Biotransport Conference*, #SB³C2015-273, Snowbird, UT, June 2015.

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.

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:

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.

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.