AWARD NUMBER: W81XWH-18-1-0525

TITLE: Mechanical Properties of the Injured CNS: Implications for Remyelination and Axonal Repair

PRINCIPAL INVESTIGATOR: Carmen Melendez-Vasquez

CONTRACTING ORGANIZATION: RFCUNY-Hunter College, New York, NY

REPORT DATE: September 2020

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
September 2020	Annual	15Aug2019-14Aug2020

4. TITLE AND SUBTITLE
Mechanical Properties of the Injured CNS: Implications for Remyelination and Axonal Repair

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-18-1-0525

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Carmen Melendez-Vasquez

E-Mail:melendez@genectr.hunter.cuny.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

RFCUNY-Hunter College
695 Park Avenue
New York, NY 10065-5024

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)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Using atomic force microscopy (AFM), we established that the mechanical properties of acute and chronically demyelinated brain lesions are distinct. We found that acute demyelinated lesions (ADL) capable of spontaneous repair are on average softer than normal appearing white matter (NAWM). By contrast, chronically demyelinated lesions (CDL), which fail to repair, are significantly stiffer than ADL and NAWM. We were also able to demonstrate that differences in myelin content and the extent of extracellular matrix (ECM) deposition are the major variables impacting tissue stiffness. Thus, in ADL, loss of myelin correlates with softening of the tissue, while remyelination correlates with recovery of tissue stiffness to levels comparable to NAWM. In CDL, augmented deposition of ECM molecules appears to be the major driver of increased stiffness. We corroborated these findings in two mouse models of demyelination and also in samples from human brain tissue obtained from multiple sclerosis patients. The results of these studies were published in Scientific Reports earlier this year.

15. SUBJECT TERMS
Multiple sclerosis, mouse models, demyelination, remyelination, tissue stiffness, extracellular matrix, atomic force microscopy

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	9	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

1. Introduction

Despite the presence of oligodendrocyte progenitor cells (OPC) capable of regenerating myelin after its loss, chronic multiple sclerosis (MS) lesions in the brain and the spinal cord are characterized by remyelination failure. Cell-based therapies involving progenitors derived from autologous stem cells are a promising approach for the treatment of chronic MS[1,2]. However, transplants delivered into CNS areas with extensive damage often result in poor cell engraftment and survival [3-5]. Recent studies have also demonstrated that mechanical cues delivered by the extracellular matrix (ECM) are capable, independently of chemical signals, of directing the differentiation of stem cell populations or promoting the differentiation of mesenchymal stem cells into specific cell types. Similarly, while soft artificial substrates mechanically similar to the healthy brain promote neurogenesis and axonal growth,[6,7], work from our laboratory and others has demonstrated that oligodendrocytes (OL), the myelinating glia of the CNS, are also mechanosensitive[8-12] and that increases in ECM stiffness inhibit their differentiation[12].

The abnormal accumulation of a wide range of ECM proteins in demyelinated lesions has repeatedly been shown to inhibit remyelination[13,14,15]. However, despite the evidence that mechanical signals may play an important role in CNS development and repair, and the fact that changes in ECM deposition resulting from injury or demyelination must change the mechanical properties of the ECM, there has been very little systematic investigation of this problem. More critically, *very little is known about how the ECM changes mechanically during the time course of disease and recovery in the CNS*. Although it is accepted that chronic demyelination ultimately causes remyelination failure[16], and that chronic and acute demyelination are associated with different types of ECM molecules deposition[13,15], there has been no systematic analysis of how demyelinating insults affect the mechanical properties of the ECM in the CNS. Attempts to measure the mechanical properties of the brain parenchyma in animal models of demyelination[17] and human aging[18] using non-invasive methods such as magnetic resonance elastography (MRE) have been limited to a macroscopic spatial resolution. Atomic force microscopy (AFM) provides an alternative that allows the examination of tissue stiffness at scales relevant to cellular mechanotransduction.

To this end we developed a protocol which allowed us to capture both optical images and AFM measurements of the brain tissue at micrometer scales. This information used in combination with immunohistochemistry (IHC) of the same region, made it possible to correlate changes in tissue stiffness with ECM structure and cellular composition of the area being examined. *This innovative approach provided unprecedented level of detail on the mechanical properties of the CNS and the mechanical stimuli experienced by specific cells populations within demyelinated lesions.* We found that acute and chronic demyelination affect the mechanical properties of CNS tissue in distinct ways when examined at cellular rather than macroscopic resolution. Specifically, acute demyelinated lesions are softer than healthy tissue, while chronic demyelinated lesions exhibit increased stiffness, which is associated with elevated ECM deposition. Thus, changes in ECM mechanical properties may be an important contributing factor to the rapid remyelination observed in acute softer lesions and the failure to remyelinate typical of chronic stiffer lesions.

2. Keywords

Multiple sclerosis, mouse models, demyelination, remyelination, tissue stiffness, extracellular matrix, atomic force microscopy.

3. Accomplishments

Major Task 1: Characterization of the mechanical properties of active and chronic demyelinating MS plaques in human brain tissue in conjunction with analysis of ECM and cellular composition

Subtask 1: Regulatory Review and approval by the USAMRMC Human Research Protection Office (HRPO).

Completed

Subtask 2: AFM and histological analysis of human MS tissue.

Completed. A total 12 human MS samples have been analyzed to date (tissue was not mechanically damaged and identifiable lesions were found). Among these six lesions from two samples were adequate for further AFM and histological studies. The results obtained from these studies were published in Scientific Reports.

The milestones for this major task 1 are completed and a paper was published in Scientific Reports. Follow-up studies will require obtaining more MS tissue from the tissue bank to further characterize the nature of the cellular and molecular changes driving the changes in stiffness in acute and chronic demyelination. An application has been submitted for these studies to both the National Multiple Sclerosis Society and NIH.

Major Task 2: Characterization of mechanical tissue properties and ECM deposition in a mouse model of demyelination

Subtask 1: Regulatory Review and approval by the USAMRMC Animal Care and Use Review Office (ACURO)

Completed

Subtask 2: Setup and maintenance of the mouse cuprizone-induced demyelination cohort.

Completed

Subtask 3: Assess the mechanical properties of the corpus callosum brains slices generated in subtask 2 via atomic force microscopy (AFM) to generate high resolution force maps of brain tissue.

Completed

Subtask 4: Perform IHC of the same regions measured via AFM to determine the extent of demyelination, oligodendrocyte differentiation and survival, monitor gliosis and deposition of extracellular matrix (ECM).

Completed

The milestones for this major task 2 were met and a paper was published in Scientific Reports.

Major Task 3: Establish the ex vivo culture system of cuprizone-demyelinated brain slices and hOPC

Subtask 1: Regulatory Review and approval by the USAMRMC Human Research Protection Office (HRPO)

Completed

Subtask 2: Induction of human OPC (hOPC) from iPSC.

Completed. We completed the expansion of 3 human cell-lines in January 2020 and have frozen stocks to be used in the pre-conditioning experiments and started their characterization in matrices of variable stiffness

Subtask 3: Pre-conditioning of hOPC prior to co-culture with mouse brain slices.

In progress: Human iPSC cultures attach and survive on hydrogels matrices of variable stiffness. After 3 weeks we are able to detect both Olig2+ (oligodendrocyte lineage) and GFAP+ (astrocytes) cells. More importantly, as predicted, we observed a change in the proportion of OPC and astrocytes present in these cultures at different stiffness. Thus, at 1.4kPa, conditions that mimic a soft acute demyelinating lesion (ADL) Olig2+ represented on average 33% of the total cells while GFPA+ made 12%. By contrast, at 5 kPa, a condition that mimics a stiff chronic demyelinated lesion (CDL) Olig2+ decreased to 19% while GFAP+ cells increased to 26%.

Subtask 4: Setup and maintenance of the mouse cuprizone-induced demyelination cohort.

Pending. These experiments could not be initiated as planed due to lab shutdown for COVID-19 in March 2020.

Subtask 5: Co-culture of hOPC on brain slice cultures.

Pending. We have tested the viability of adult mouse brain slices and determined they can be maintained *ex-vivo* for up to a week. We have not started to culture human-iPSC in these cultures as we need to obtain sufficient numbers.

Subtask 6: IHC analysis of co-cultures and data analysis.

Subtasks 4-6 are still pending. Due to laboratory closure we were unable to initiate the studies with the cuprizone model and hOPC.

Pending.

Major task 3 is still ongoing. Ace Alcantara a PhD student recruited last year is the person directly working on this task a spart of his thesis project. He was fully trained by Dr. Urbanski.

4. Impact

We have provided novel insights regarding micro-scale changes in the mechanical properties of acute and chronic demyelinating MS lesions, and how these changes might correlate to remyelination failure in advance stages of disease. These findings have the potential to help optimize the methods currently used to generate human OPC for autologous transplantation using iPSC technology, as well as guide the efforts for the efficient targeting and delivery of these cells to MS patients.

5. Changes/Problems

The laboratory has to be closed due to the COVID-19 on March 13, 2019. This delayed significantly our research progress, especially for major task 3, sub-task 4-6 which require animal work. We have requested a 12-month extension to complete these experiments. Laboratories at Hunter College have been allowed to resume research (starting in mid-July) and we are planning to re-initiate this work soon.

6. Products

Urbanski MM, Brendel MB, Melendez-Vasquez CV (2019). Acute and chronic demyelinated CNS lesions exhibit opposite elastic properties. *Sci Rep.* 2019 Jan 30;9(1):999. doi: 10.1038/s41598-018-37745-7.

Alcantara, A.; Urbanski, M.; Parikh, D.; Wang, H.; Melendez-Vasquez, C. Role of Mechanotransduction in the differentiation of human oligodendrocytes . Poster. Glia in Health and Disease 2020 Virtual Poster Session. Cold Spring Harbor Laboratory Meeting July 16-July 19, 2020

7. Participants and other collaborating organizations

Name:	*Carmen Melendez-Vasquez*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	*0000-0001-8752-8956*
Nearest person month worked:	*1 CM*
Contribution to Project:	*Reviewed data. Manuscript preparation. Prepared report.*

Name:	*Mateusz Urbanski*
Project Role:	*Research Associate*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*12 CM*
Contribution to Project:	Carried out experiments for Major Task 1. Trained a graduate student assisting in the project.

Name:	*Ace Alcantara*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*3 CM*
Contribution to Project:	*In charge of experiments in major task 3*
Funding Support:	*NIH-NIGMS RISE Program*

No changes in active support or other support for the PI since the last reporting period.

No other organization involved.

8. Special Reporting Requirements

N/A

9. Appendices

References

1 Douvaras, P. & Fossati, V. Generation and isolation of oligodendrocyte progenitor cells from human pluripotent stem cells. *Nature protocols* **10**, 1143-1154, doi:10.1038/nprot.2015.075 (2015).

2 Douvaras, P. *et al.* Efficient generation of myelinating oligodendrocytes from primary progressive multiple sclerosis patients by induced pluripotent stem cells. *Stem cell reports* **3**, 250-259, doi:10.1016/j.stemcr.2014.06.012 (2014).

3 Mahmood, A., Lu, D., Wang, L. & Chopp, M. Intracerebral transplantation of marrow stromal cells cultured with neurotrophic factors promotes functional recovery in adult rats subjected to traumatic brain injury. *Journal of neurotrauma* **19**, 1609-1617, doi:10.1089/089771502762300265 (2002).

4 Harting, M. T., Sloan, L. E., Jimenez, F., Baumgartner, J. & Cox, C. S., Jr. Subacute neural stem cell therapy for traumatic brain injury. *J Surg Res* **153**, 188-194, doi:10.1016/j.jss.2008.03.037 (2009).

5 Ma, H., Yu, B., Kong, L., Zhang, Y. & Shi, Y. Transplantation of neural stem cells enhances expression of synaptic protein and promotes functional recovery in a rat model of traumatic brain injury. *Mol Med Rep* **4**, 849-856, doi:10.3892/mmr.2011.510 (2011).

6 Engler, A. J., Sen, S., Sweeney, H. L. & Discher, D. E. Matrix elasticity directs stem cell lineage specification. *Cell* **126**, 677-689, doi:S0092-8674(06)00961-5 [pii]10.1016/j.cell.2006.06.044 (2006).

7 Georges, P. C., Miller, W. J., Meaney, D. F., Sawyer, E. S. & Janmey, P. A. Matrices with compliance comparable to that of brain tissue select neuronal over glial growth in mixed cortical cultures. *Biophys J* **90**, 3012-3018, doi:S0006-3495(06)72482-1 [pii]10.1529/biophysj.105.073114 (2006).

8 Jagielska, A. *et al.* Mechanical Strain Promotes Oligodendrocyte Differentiation by Global Changes of Gene Expression. *Frontiers in cellular neuroscience* **11**, 93, doi:10.3389/fncel.2017.00093 (2017).

9 Lourenco, T. *et al.* Modulation of oligodendrocyte differentiation and maturation by combined biochemical and mechanical cues. *Scientific reports* **6**, 21563, doi:10.1038/srep21563 (2016).

10 Hernandez, M. *et al.* Mechanostimulation Promotes Nuclear and Epigenetic Changes in Oligodendrocytes. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **36**, 806-813, doi:10.1523/JNEUROSCI.2873-15.2016 (2016).

11 Shimizu, T. *et al.* YAP functions as a mechanotransducer in oligodendrocyte morphogenesis and maturation. *Glia* **65**, 360-374, doi:10.1002/glia.23096 (2017).

12 Urbanski, M. M. *et al.* Myelinating glia differentiation is regulated by extracellular matrix elasticity. *Scientific reports* **6**, 33751, doi:10.1038/srep33751 (2016).

13 Back, S. A. *et al.* Hyaluronan accumulates in demyelinated lesions and inhibits oligodendrocyte progenitor maturation. *Nature medicine* **11**, 966-972, doi:nm1279 [pii]10.1038/nm1279 (2005).

14 Lau, L. W. *et al.* Chondroitin sulfate proteoglycans in demyelinated lesions impair remyelination. *Ann Neurol* **72**, 419-432, doi:10.1002/ana.23599 (2012).

15 Stoffels, J. M. *et al.* Fibronectin aggregation in multiple sclerosis lesions impairs remyelination. *Brain : a journal of neurology* **136**, 116-131, doi:10.1093/brain/aws313 (2013).

16 Frohman, E. M., Racke, M. K. & Raine, C. S. Multiple sclerosis--the plaque and its pathogenesis. *The New England journal of medicine* **354**, 942-955, doi:354/9/942 [pii]10.1056/NEJMra052130 (2006).

17 Schregel, K. *et al.* Demyelination reduces brain parenchymal stiffness quantified in vivo by magnetic resonance elastography. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 6650-6655, doi:10.1073/pnas.1200151109 (2012).

18 Arani, A. *et al.* Measuring the effects of aging and sex on regional brain stiffness with MR elastography in healthy older adults. *NeuroImage* **111**, 59-64, doi:10.1016/j.neuroimage.2015.02.016 (2015).