Alteration of Oligodendrocyte Functional Mechanics By Neurodegenerative Disease
Kamilah Y. Amen, Nicholas J. Braun, Patrick W. Alford
Department of Biomedical Engineering, University of Minnesota

BACKGROUND
• Neurodegenerative diseases affect one billion people worldwide and alter the function and properties of the cells they affect
• Both amyloid beta-1-40 (Aβ-1-40) & amyloid beta-1-42 (Aβ-1-42), and alpha-synuclein (α-synuclein) are proteins overactive in Alzheimer’s and Parkinson’s disease, respectively
• Neurons in the central nervous system are targets of these proteins
• Neurons are protected and supported by surrounding glial cells
• Oligodendrocytes are glial cells that produce myelin sheaths, wrapping around neuronal axons, protecting the axons and strengthening action potentials
• The interactions between the aggregated proteins and oligodendrocytes and the effects on the traction force are unknown
• We use traction force microscopy (TFM) and immunofluorescent staining (IF) to explore how disease related extracellular factors alter cellular force generation and morphology

EXPERIMENTAL METHODS
Cell Substrate Construction
• Ultra violet ozone (UVO) radiation was applied to the 25mm coverslips for 8 minutes to sterilize the surface
• Bind-silane applied onto surface of 25mm coverslips until dry
• 15mm coverslips were cleaned, plasma treated, and treated with poly-D lysine (PDL) for 30 minutes
• 2.5 kPa polyacrylamide gel was fused with fluorescent beads that measured the movement of the cells' appendages in TFM
• 10 μL of gel was placed onto 25mm coverslips and covered with PDL treated 15mm coverslips to cure for 1 hour
• After 15mm coverslips are removed, gels remain attached to 25 mm coverslips and treated with 4% bovine serum albumin (BSA)

RESULTS
• Oligodendrocyte cells were harvested from neonatal rat pups by Braun, N
• Cells were kept in an incubator at 37°C and in standard culture medium; media was changed every two days
• Cells were treated with 1μM of proteins (Aβ-1-40, Aβ-1-42, and α-synuclein) 24 hours before TFM was performed
• Cells were seeded onto the gels and allowed to adhere for 24 hours

Traction Force Microscopy (TFM)
• All experiments were performed using an Olympus IX81ZDC confocal and Zeiss Axiom Observer inverted microscope at 40X magnification
• Gel-embedded fluorospheres were imaged with the cell intact
• Cell was lysed from the gel using sodium dodecyl sulfate (SDS) and gel embedded beads were imagined again
• Cell traction forces were measured using previously determined methods

Immunofluorescent (IF) Staining
• Cells were fixed with 4% paraformaldehyde for 5 minutes
• Phallolidin and DAPI, in a 1:200 dilution with 0.5% BSA in 1X phosphate-buffered saline (PBS), were used to label actin and chromatin, respectively
• Cells were imagined using an Olympus IX81ZDC confocal and Zeiss Axiom Observer inverted microscope at 40X magnification

DISCUSSION
• Glial cells provide the substrate on which neurons exist and function
• Our data suggest that Aβ-1-40, Aβ-1-42, and α-synuclein alter the traction force generated by oligodendrocytes
• This increase in force could indicate disturbances in neuronal function and mediate disease progression

REFERENCES