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Manipulation of extracellular matrix mechanical cues to stimulate oligodendrocytes to promote remyelination

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SUMMARY

The purpose of this project is to test whether extracellular matrix (ECM) mechanical cues can be used to stimulate oligodendrocytes to proliferate quicker and to differentiate into myelinating cells in greater amounts. This was to combat symptoms present with Multiple Sclerosis (MS), a demyelinating disease in which the immune system attacks the myelin sheath. It was hypothesized that the softest gel of the three would have the most significant increase in oligodendrocyte proliferation and differentiation. To culture these cells, a 2D cell coating hydrogel system was implemented, with ratios of 1:1 (the control), 1:3, and 3:1 of gel to dilution solution. To test proliferation both manual cell counts and an MTT assay were performed. The results demonstrated that there was a significant difference in proliferation rates between the control (gel with a ratio of 1:1) and the variable (gels with a ratio of 1:3 and 3:1) samples, with the variable gels having a higher proliferation rate than the control. However, there was not a significant difference between the two variable samples. To test the impact of the differing gels on differentiation of oligodendrocytes into myelinating cells, expression of the PLP and CNPase proteins, which are known to be present during the first couple days of differentiation, was assessed using immunocytochemistry. The results displayed that the cells seeded on the softer gel (1:3 ratio) had higher levels of PLP and CNPase proteins, although to a lesser extent for the latter protein, compared to the cells seeded on the control and other variable gels. Overall, the results showed that manipulating the stiffness of the ECM increased both aspects, and that the softer gels had the greatest impact on differentiation, but not proliferation.

INTRODUCTION

Multiple Sclerosis (MS) is a disease in which the body's immune system attacks the myelin sheath and therefore disrupts communication in the central nervous system (CNS), resulting in symptoms such as inability to walk, blurred vision, and tremors (1). One of the main problems of MS is that the disease decreases the ability for the myelin sheath to regenerate. To counteract this degeneration, several concepts have been studied to speed up the process of remyelination, such as cytokine-based immune-intervention and recombinant monoclonal antibody-induced remyelination (2). Despite these different avenues, no therapies have been successful in treating the disease, rather, they solely treat the symptoms that follow disease onset. For this reason, MS remains a significant disease in neuroscience due to the degradation of the myelin sheath still being heavily prominent in those who suffer from the disease.

The myelin sheath is an essential part of the CNS; thus, its degradation is detrimental in disease conditions. Myelin sheath is composed of a modified plasma membrane which is used to provide protection and insulation around the axon of the neuron. The process of myelination stems from the folding, elongation, and deposition of myelin through the use of oligodendrocytes (OLs) within the CNS (3). OLs originate from the ventral ventricular zone, in which their precursors, oligodendrocyte precursor cells (OPCs), migrate up the spinal cord until differentiating into the OLs that could differentiate further into myelinating OLs (4). There are a variety of proteins that help with different aspects of OL myelination. One of the first proteins that is seen in OLs during myelination is 2',3'-Cyclic- nucleotide 3'-phosphodiesterase (CNPase). Though the specific biological function of this protein is unknown, it has been found to play a critical role in the beginning steps of the formation of the myelin sheath since it can mainly be found in OLs during this myelination more so than other cell types (5). Another protein found during the process of myelination is Proteolipid Protein (PLP). This is one of the proteins responsible for maturing the OLs, so that they have the ability to create and maintain the structure of the myelin sheath (6). Due to the role of PLP in the human body, it is often more naturally occurring than CNPase within this biological system (6). Once the myelin sheath has been formed, myelin oligodendrocyte glycoprotein (MOG) becomes a target antigen for immune-mediated demyelination through its display on the surface of OLs and is potentially one of the causes of demyelinating diseases, such as MS (7).

With the current treatments and research available for MS, one aspect that has not been looked into thoroughly is the use of extracellular matrix (ECM) mechanical cues. These cues have been found to stimulate multiple processes within the cell, such as proliferation, metabolism, and differentiation (8). The ECM plays a crucial role in many processes of different types of cells due to the vast number of proteins and signaling molecules it contains. One such process for OLs is oligodendrogenesis, which is part of the process of differentiation to generate new OLs and is controlled by factors in the ECM such as signaling molecules and proteins (9). Through processes like these, the ECM has control over many of the aspects of the myelination process. There are also differences seen between ECM around OLs compared to other cell types within the body. Along with this, the brain contains softer ECM, and the manipulation of ECM leads to the

alteration of many different biological factors (8). One group of these factors are called mitogens, which control aspects of cell division by relieving factors inhibiting the progress of the cell cycle (10). Additionally, many of these factors aid with the differentiation and proliferation of a plethora of other cell types, including stem cell differentiation, the onset of some types of cancers, regeneration of cartilage, and many others.

In order to mimic the ECM and the control that it has over the biological processes stated above, a polysaccharide-based hydrogel was used as an alternative. This hydrogel was modified with the arginylglycylaspartic acid (RGD) peptide; thus, the mechanical properties of the matrix can be modified to meet the varying conditions of this experiment, in which the standard set by the company VitroGel™ is through the ratio 1:1, gel to dilution solution (11). These mechanical properties are adjusted through the different concentrations of the hydrogel, which affects the ionic concentration of the medium, thus creating the vast range of strength and stiffness of the hydrogel (11). Similarly, with this hydrogel, the changes in ionic concentration affects different chemicals and molecules found within the gel components, thus allowing this system to effectively mimic some of the key aspects that help to promote or limit certain biological functions, such as proliferation or differentiation, within cells (11). This differs from other methods used for measuring the stiffness of hydrogels, such as kPa and AFM-based measurements (12). However, another common gel type that can be used to culture cells in an *in vivo* environment, or one extremely close to that, is a polyacrylamide hydrogel, in which this gel is known for its use in protein separation during gel electrophoresis (13). With this being said, the polysaccharide gel has a wide array of uses in drug-delivery and can therefore be used with a wider variety of cell cultures and mediums, which was the main reason for its use in this project.

To detect the proteins in OLs when the ECM mechanical cues are being manipulated a variety of different assays can be used. One assay is Immunocytochemistry (ICC) staining, in which cells are fixed, blocked, permeabilized and incubated with the respective antibodies in order to detect a protein using fluorescence (14). ICC staining allows for the researchers to see in greater detail the differences between the proteins in the sample with less cost. Similarly, more analysis (both qualitative and quantitative) can be done through the images and because of this provides more support for or against the researcher's hypotheses, which in return provides sufficient data to draw conclusions about the functionality of the oligodendrocytes.

In order to see whether or not the mechanical cues from the ECM increase proliferation several assays can be used. One such assay is a manual cell count, in which cells are counted on a grid using a hemocytometer (15). Another assay used is an MTT assay, which looks for mitochondrial activity in order to see how many viable cells there are in a sample, since mitochondrial activity is associated with viable cells (16). With these, both are valid assays that can be used to see change in proliferation as they give heavily quantitative data. Although, in this project, the MTT assay was primarily used to analyze this change and the manual cell count used as a way to ensure appropriate density before plating the cells.

Overall, this project was developed to test the validity of the following two hypotheses: 1) if there would be an increase in proliferation of oligodendrocytes this would result from the decrease in stiffness of the surrounding ECM and 2) if the promotion of the differentiation of oligodendrocytes is recorded, it is due to a decrease in the stiffness of the hydrogel used to mimic the ECM. It was with these hypotheses that results showed support of the latter but not the first. This demonstrates that the manipulation of the ECM affected the differentiation of OLs more so than their proliferation.

RESULTS

The results below were gathered using MTT assays and ICC staining, which were used in order to provide an accurate quantification of the number of proliferating cells within the samples (MTT) and the abundance of each protein being tested within the different samples (ICC). Prior to the staining of the cells through ICC the effect of the differing environmental conditions can be seen clearly through imaging of the cell samples. The samples in the imaging chambers resulting from growth in the 1:3 gel cover more surface area and are available in a greater quantity (**Figure 1a**) than the differing groups. Increased surface area coverage and quantity can also be seen with the samples taken from the 3:1 gel (**Figure 2a**) although less than the prior. These are both in comparison to the control gel (1:1), where there is cell growth but much less than the variable groups (**Figure 3a**). These data were taken after the same amount of days, to ensure the same growth time and a manual cell count was performed to quantify the amount of cells shown. With this being said, the cells were each grown until they reached 70% confluency in the chambers before their use for ICC. This was to ensure that each chamber for the ICC assay had the same density of cells for staining and was quantified by using a manual cell count.

To test the ability of differing gel stiffnesses to impact OL differentiation, quantification of the PLP and CNPase proteins was conducted using fluorescent imaging. For the OLs plated on the soft gel (1:3), there was a much greater amount of PLP protein (red) compared to the CNPase protein (green) (**Figure 1b and c**). Additionally, the OLs plated on the stiff gel (3:1) also had PLP protein (red) dominating the CNPase protein (green), although less so than for the soft gel (**Figure 2b and**

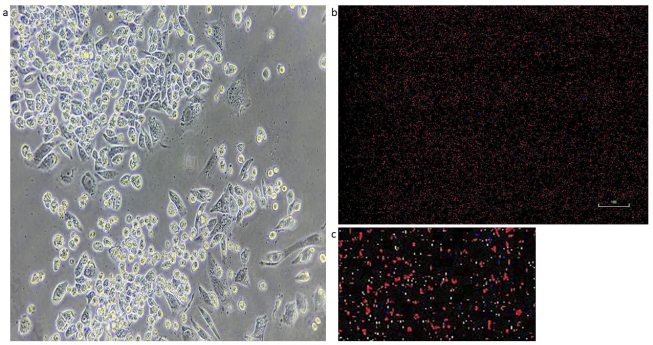

Figure 1: Oligodendrocytes cultured in a 1:3 dilution of gel to dilution solution. (a) Oligodendrocytes were fixed during the Immunocytochemistry (ICC) protocol. Pictures were taken with the Nikon inverted microscope at 100 microns. (b) Oligodendrocytes were fixed and stained during the Immunocytochemistry (ICC) protocol. Pictures were taken with the Nikon inverted microscope with the fluorescence attachment at 100 milliseconds exposure time. Where red is the Proteolipid protein and green is the CNPase antibody. The scale bar represents 100μm. (c) Selected portion of Figure 1b for clarity of PLP and CNPase expression.

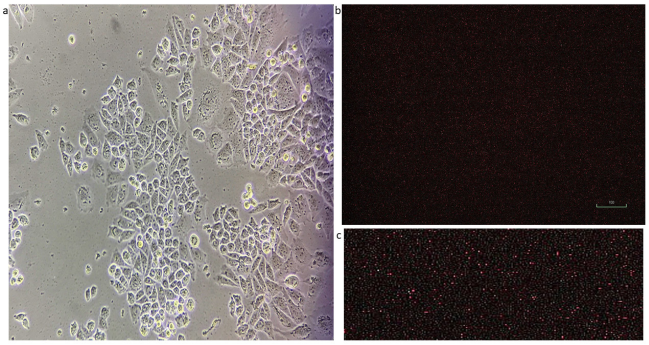

Figure 2: Oligodendrocytes cultured in a 3:1 dilution of gel to dilution solution. (a) Oligodendrocytes cultured in a 3:1 dilution of gel to dilution solution were fixed during the Immunocytochemistry (ICC) protocol. Pictures were taken with the Nikon inverted microscope at 100 microns. (b) Oligodendrocytes cultured in a 3:1 dilution of gel to dilution solution were fixed and stained during the Immunocytochemistry (ICC) protocol. Pictures were taken with the Nikon inverted microscope with the fluorescence attachment at 100 milliseconds exposure time. Where red is the Proteolipid protein and green is the CNPase antibody. The scale bar represents 100µm. (c) Selected portion of Figure 2b for clarity of PLP and CNPase expression.

c). By contrast, the fluorescence imaging of the OLs plated on the control gel (1:1) displayed no significant concentration of the CNPase protein or PLP protein (**Figure 3b and c**). The increasing expression of PLP protein on the soft (1:3) and stiff (3:1) gels, as compared to control, demonstrated that altering the stiffness of the ECM had an increase in the differentiation of OLs (p = 3.183E-5 for 1:3 compared to 1:1 and p= 1.839E-5 for 3:1 compared to 1:1), with the soft gel having more of an effect than the stiff gel (p = 0.000769 for 1:3 compared to 3:1). These were drawn from the comparison between the variable and control gels and between the variable gels.

Thus, the hypothesis for OL differentiation is supported, with the two variable samples having higher PLP protein as

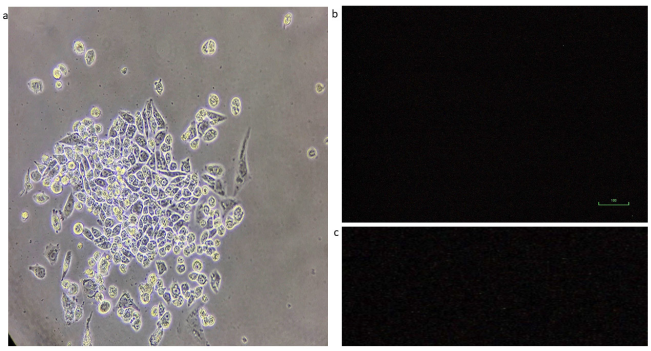

Figure 3: Oligodendrocytes cultured in a 1:1 dilution of gel to dilution solution. (a) Oligodendrocytes cultured in a 1:1 dilution of gel to dilution solution were fixed during the Immunocytochemistry (ICC) protocol. Pictures were taken with the Nikon inverted microscope at 100 microns. (b) Oligodendrocytes cultured in a 1:1 dilution of gel to dilution solution were fixed and stained during the Immunocytochemistry (ICC) protocol. Pictures were taken with the Nikon inverted microscope with the fluorescence attachment at 100 milliseconds exposure time. Where red is the Proteolipid protein and green is the CNPase antibody. The scale bar represents 100µm. (c) Selected portion of Figure 3b for clarity of PLP and CNPase expression.

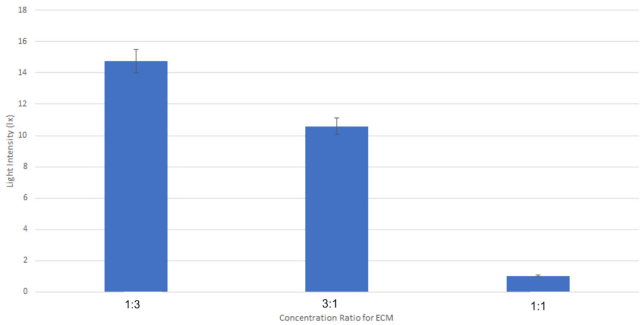

Figure 4: Average Light Intensities for PLP and CNPase Antibody Expression. Oligodendrocytes were cultured in hydrogels with varying stiffnesses and then the cell metabolic activity was measured using the 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide assay (MTT assay). Each bar represents an average ten samples for each trial performed with the MTT assay. Error bars represent the standard error of the mean for each data set. Experimental groups were compared via t-test to each other and to the control: = $p < 0.05$

compared to the control due to changes in stiffness. Likewise, this also supports the hypothesis that the softer gel, since it is more similar to the stiffness of brain ECM, has a greater impact on OL differentiation (**Figure 4**).

Next, to test the ability of differing gel stiffnesses on the impact of OL proliferation, an MTT assay was used to compare the proliferation rates of cells in the control gel (1:1) to cells in the variable gels (either 1:3 or 3:1) (**Figure 5**). Both of the variable samples demonstrated a significantly greater proliferation rate than the control (p = 7.837E-5 for 1:3 compared to 1:1 and p = 9.219E-6 for 3:1 compared to 1:1), although there was not a significant difference when the variable samples were compared to each other (p = 0.463 for 1:3 compared to 3:1) (**Figure 5**). With this, it was determined that the change in stiffness of the ECM had an increased effect on the OLs' proliferation, which supports the general hypothesis, but the hypothesis of the softer gel influencing the cells' proliferation to a greater extent was not supported.

DISCUSSION

The first hypothesis, that if extracellular matrix mechanical cues are used to promote differentiation in OLs, then an increase in the number of differentiated cells will be seen with decreased stiffness of the hydrogel, was supported by the ICC assay showing an increase of the CNPase and PLP protein expression. Here, it was displayed that the softer gel had a significantly greater effect on the differentiation of the cells than the harder and control gels (p= 0.000769 for 1:3 compared to 3:1and p= 3.183E-5 for 1:3 compared to 1:1). However, the majority of protein expressed through ICC was the PLP protein and not the CNPase protein, as these were expected to be displayed in relatively equal amounts. This could be due to the PLP protein being a naturally more frequently occurring protein in the human body or that the gel only aided in increasing the expression of PLP, although the former is more likely. Another analysis pertaining to the difference between expression could've been the timeframe in which the assays were carried out. More specifically, CNPase can start to appear a day or more after the point where PLP begins to be present. Although a time frame was set to observe the cells where it was thought that both would

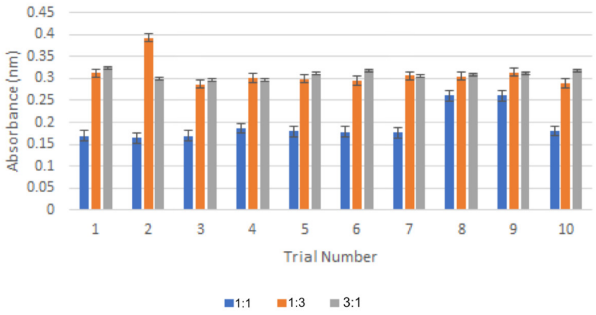

Figure 5: Absorbance Per Trial for MTT Assay. Oligodendrocytes were cultured in hydrogels with varying stiffnesses and then tagged with fluorochrome antibodies to see protein expression via Immunocytochemistry (ICC). Each bar represents an average of five areas measured for light intensity using images taken with ICC. Error bars represent the standard error of the mean for each data set. The experimental groups were compared via t-test to each other and to the control: = $p < 0.05$

be present in considerable amounts, more time may have been needed to see more significant amounts of CNPase.

Secondly, proliferation was tested to see if this value will increase with a decrease in ECM stiffness and it was found that this is partially supported since the alteration of the stiffness of gels resulted in an increase in proliferation. This was demonstrated through the MTT assay, which displayed a significant difference between the variables and the control but not between the two variables. The hypothesis that the softer gel would significantly lead to a greater proliferation rate than the harder gel was not supported. This could have been from the difference in the ratios of gel not having a wide enough gap between them or that the same cues are exhibited whether the gel is harder or softer.

However, the hypothesis stating that if extracellular matrix mechanical cues are used to increase proliferation in oligodendrocytes then the reproduction rate would increase with a decrease in stiffness of the hydrogel, was not completely supported due to the absence of a significant difference between the two variable samples. It was hypothesized that the softer gel would significantly lead to a greater proliferation rate than the harder gel. With the p-values between the two groups being higher than 5% and the error bars overlapping, it's seen that a significant difference is not the case.

This difference between results and the hypothesis could be due to a number of reasons, one of which being that the difference from the ratio of gel to dilution solution in the hard versus the soft gel is not being great enough to see statistically different results. Aside from the physical set up of the assays, another reason that might have led to these results is the similarities between the different stiffnesses of gel and the response from the cells being studied. More specifically, in conjunction to there not being an extreme difference in the ratios for each variable group, the response that the cells had in general to a change in the environments they were growing in could have led to the similarity among the results within some of the assays. The MTT assay was performed once the cells had reached confluency in their respective environments, leading to the conclusion that the change in the results seen between each of the variable groups and overall could be the initial result of the cells changing to adapt to their new environment. This would explain that with the second hypothesis there was a greater statistical difference between the variable groups, as there was more time for each of the cell groups to adapt to their new environment.

One of the major issues that occurred in this project was the difficulty in sourcing and beginning a culture of oligodendrocytes, in which several attempts were made initially to account for cells delivered thawed and infected. However, new vials provided ample healthy cells to start the culture. Additionally, during the ICC protocol the counterstain that was used remained in the cell imaging chambers instead of being taken out due to an absence of instruction in the methods; this caused some of the cells to lift and the fluorescence light to refract off of the counterstain, although images were able to be taken and analyzed. However, this led to solely grouped image analysis being possible, of both PLP and CNPase, rather than separately as well.

The main purpose of this project was to provide insight into Multiple Sclerosis (MS), a disease in which the myelin sheath in the CNS is attacked by the body's immune cells. With this, the project looked into a method that would help ease the symptoms that the patients who suffer from MS have by increasing the amount of myelin sheath that could be made in a given time period. The rationale that increasing the proliferation and differentiation rate to the same rate at which the myelin sheath is being degraded in MS would slow or even halt the expression of disease symptoms. This method could be easily applied *in vivo* because hydrogel is made so that it is easily injectable and does not solidify to its stiffness immediately, meaning that it could be administered to patients with relative ease. Although this research is specified to MS, the applications extend to other areas in medicine that would require the alteration of mechanical cues of the ECM to induce changes in surrounding cells.

To look back upon previous studies done within the field throughout this project has provided valuable information for the resulting path taken by the researchers. One of the key choices altered by this was through the differences between the types of hydrogel systems found within research. With the prior work done, the system used followed that of a polyacrylamide hydrogel (12)(13), which has been described in detail during the introduction. Although, with the research conducted for this paper a polysaccharide hydrogel was used as it better fit the parameters of this project. It is important to note, however, the findings of the studies using this alternate hydrogel, as they further support those found within this paper. One of which looks at the applications of the combined effects of biochemical and biophysical elements found to alter certain cellular functions (13), which highlights the importance of both of these aspects and their work towards cells. The use of these components together has worked to stimulate the question of their use independently, which was studied during this paper. Alongside this, the polyacrylamide gel has been used to display how OPCs are mechanosensitive, meaning that their cues can be manipulated in ways that are similar to the ways used with other cell types (12). This study also helps to support the findings in this paper as with the information of this cell type being mechanosensitive they are more likely to be susceptible to this manipulation of their mechanical cues, which was looked at throughout this project.

Future research would include increasing the ratios between the different concentrations of gel, to see if a greater

difference in stiffness would lead to a greater difference in impact on proliferation and differentiation. It would also be useful to look at the expression levels of different proteins, to see if others are more readily expressed than the ones tested here, such as Myelin Basic Protein (MBP). Lastly, it would be imperative to test this rationale on a larger scale model, where the differentiation into myelinating oligodendrocytes would more clearly be seen. A model akin to an *in vivo* approach (more so than the hydrogel), where the formation of axons can be present and the elongation quantified would be best suited. Examples of larger scale models could include that of within organisms such as mice, but also within an environment where axons could be stimulated into growth. This was not present in this experiment, as axon elongation comes much later in the myelination process and the proteins researched appear towards the beginning of this process.

MATERIALS AND METHODS

The Oligodendrocyte cell line was murine-derived from Celprogen™.

2D cell coating culture with VitroGel hydrogel system and recovery

A sterile 15mL tube was prepared for mixing and the VitroGel (VitroGel™ -TheWell Bioscience) was pipetted into the tube (amount depended on concentration; 0.5mL gel for 1:1, 0.5mL gel for 1:3, and 1.5mL gel for 3:1). The concentration of VitroGel was then adjusted by diluting it with the dilution solution (0.5mL solution for 1:1, 1.5mL solution for 1:3, and 0.5mL solution for 3:1). This mixture was then added to cell culture medium (Celprogen™), mixed, and transferred to a 24-well plate (0.25mL medium for 1:1, 0.5mL medium for both 1:3 and 3:1, this was to ensure the same quantity went into each well). The plate was gently tilted and swirled to ensure an even covering of the hydrogel at the bottom of each well. After 15 minutes, the gel had formed and attached to the plate, and OLs were added at the desired density on top of the hydrogel (OLs were taken from a T25 culture at approximately 70% confluency). Once cells had reached desired density (approximately 70% confluent in each well), a dry bath was set to 37 degrees Celsius and a 15mL centrifuge tube was prepared for cell recovery. 6ml of VitroGel recovery solution (VitroGel™ -TheWell Bioscience) was added to the tube and the solution was warmed to 37 degrees Celsius. Once the medium covering the top of the hydrogel was removed from the well plate, 1ml of warm recovery solution was added to each of the wells and a sterile spatula was used to scoop the detached hydrogel, which was then placed into the tube with the rest of the warm recovery solution. The tube was then capped and inverted 20 times before incubation in the water bath for 2-3 minutes. This was then repeated 3 times and the solution was centrifuged at 9000 rpm for 7 minutes. The supernatant was discarded and the pellet was collected for further testing.

Cell Counting Assay

Sterile cell culture technique was maintained, and cells were grown at 37 degrees Celsius. Cells were checked under a microscope (Nikon Eclipse ts100 inverted microscope at 100x) for any signs of bacterial or fungal contamination. Existing media was removed, and flasks were washed with 2-5mL of phosphate buffer saline (PBS). 5 mL of culture medium was added to the cell culture flask, and the cells were detached using a sterile cell scraper. Cells were centrifuged for 5 minutes at 1200 RPM and then the supernatant was removed. Cell pellets were then resuspended with 5mL of culture medium, and 0.5mL was taken and transferred into a sterile Eppendorf tube. Cells were diluted in Trypan Blue in a 1:5 dilution. 100µL of the Trypan Blue/cell suspension solution was taken and carefully pipetted into the counting well of a hemocytometer where cells were viewed under a 10X microscope. The cell concentration was found by taking the average number of viable cells in the 4 sets of 4x4 grids and multiplying that by 10000 to get the number of cells per mL. This was then multiplied by 5 to correct for the 1:5 dilution of the Trypan Blue. This assay was replicated three times for each of the data sets in the experiment.

MTT Assay

The assay kit was purchased from Cayman Chemical. First, the assay buffer was prepared by dissolving the cell-based assay buffer tablet in 100mL of distilled water. To prepare the MTT reagent, the MTT reagent powder (25mg) was dissolved in 5mL of assay buffer by vortexing. The cells were seeded in a 96-well plate at a density of $5x10^2$ -10^5 cells per well (range suggested by Cayman Chemical) in 100µL of culture medium and then incubated for 24 hours. After 10µL of the MTT reagent was added to each well, and the solution was mixed gently on an orbital shaker for 1 minute. The cells were then incubated for 3-4 hours at 37 degrees Celsius in an incubator. Once the allotted incubation time had ended, 100µL of crystal dissolving solution was added to each well and then incubated once more for 4-18 hours in an incubator. The absorbance was then measured at 570nm using a microplate reader (SpectraMax Plus 384 -Molecular Devices).

Immunocytochemistry for Fluorescence Staining

Cultured cells were grown on cell imaging chambers for three days at 37 degrees Celsius. Cells were then rinsed in PBS and incubated in 1% Paraformaldehyde in PBS for 10 minutes at room temperature. After the cells were rinsed three times with PBS, a permeabilizing solution (Thermo Fisher Scientific GAS002S-5) was added and then incubated for 10 minutes at room temperature. Once the cells had again been rinsed in PBS three times, the sample was blocked with 1mL of blocking buffer (Thermo Fisher Scientific 37579) at room temperature for 30 minutes. While the samples were being blocked, the primary antibodies (Boster Biological Technology A01056-1 and Neuromics CH23013) were diluted to 1:2500-1:5000 (range for both antibodies) in blocking buffer and then added at 500µL per well after the incubation. Once the antibody was added, the samples were incubated overnight at 4 degrees Celsius. The samples were washed once in PBS, incubated with wash buffer for 5-10 minutes, and then washed in PBS two more times. During this incubation, the fluorochrome-conjugated secondary antibodies (Abcam ab150169 and Santa Cruz Biotechnology Inc. sc-516251) were diluted to the same dilution as previously stated in blocking buffer with 500µL added to each well. After a 1-hour incubation at room temperature in the dark, the samples were washed with PBS and then incubated in wash buffer for 5-10 minutes. Once the samples had then been washed two more times in PBS, 300µL of DAPI counterstain was added

to each well and incubated at room temperature in the dark for 1-5 minutes. Once the counterstain had been removed, cells were looked at under fluorescence microscope (Nikon Eclipse ts100 inverted microscope at 100x with fluorescence attachment).

Statistical Analysis

A *t*-test was used to compare the two variable groups to each other and to the control with the data from the MTT assay. Standard error was also found to produce error bars in the graph displaying the data for the MTT assay. The pictures from the fluorescence staining were analyzed using the computer software ImageJ (19), where the light intensities of different sections of ICC images were measured and then averaged. A *t*-test was then used to once again compare the statistical difference between the variable groups and the control for light intensity.

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REFERENCES

1. "Multiple Sclerosis." *Mayo Clinic*, www.mayoclinic.org/diseases-conditions/multiple-sclerosis/symptoms-causes/syc-20350269.
2. Podbielska, Maria, et al. "Myelin Recovery in Multiple Sclerosis: The Challenge of Remyelination." *MDPI*, doi:10.3390/brainsci303128.
3. Morell, Pierre, and Richard H. Quarles. "The Myelin Sheath." *National Center for Biotechnology Information*, www.ncbi.nlm.nih.gov/books/NBK27954/.
4. Bradl, Monika. "Oligodendrocyte: Biology and Pathology." *Springer*, doi:10.1007/s00401-009-0601-5.
5. "Family: CNPase (PF05881)." *Pfam*, pfam.xfam.org/family/CNPase.
6. Nadon, NL, and M. West. "Myelin proteolipid protein: function in myelin structure is distinct from its role in oligodendrocyte development." *National Center for Biotechnology Information*, DOI:10.1159/000017354.
7. "MOG myelin oligodendrocyte glycoprotein [Homo sapiens (human)]." *PubMed*, www.ncbi.nlm.nih.gov/gene/4340.
8. Janson, Isaac A., and Andrew J. Putnam. "Extracellular matrix elasticity and topography: material-based cues that affect cell function via conserved mechanisms." *U.S. National Library of Medicine*, doi:10.1002/jbm.a.35254.
9. Khaksar, Zabihollah, et al. "The Role of Extracellular Matrix in Myelination and Oligodendrogenesis of the Central Nervous System." *Research Gate*, DOI:10.29252/shefa.7.2.66.
10. Alberts, Bruce, et al. *Molecular Biology of the Cell*. 4th ed., Garland Science. *National Center of Biotechnology Information*, Garland Science,www.ncbi.nlm.nih.gov/books/NBK26877/.
11. "VitroGel." *TheWell Bioscience*, pp. 1-22.
12. Jagielska et al. (2012) Mechanical Environment Modulates Biological Properties of Oligodendrocyte Progenitor Cells. Stem Cells Dev. 10.1089/scd.2012.0189
13. Lourenço et al. (2016) Modulation of oligodendrocytes differentiation and maturation by combined biochemical and mechanical cues. Sci. Rep 10.1038/srep21563
14. *A Guide to Protein Detection*. docs.abcam.com/pdf/proteins/a-guide-to-protein-detection.pdf.
15. "Manual cell counting V.S. Automated cell counting." *ChemoMetec*, chemometec.com/manual-cell-counting/.
16. Meerloo Van, J., et al. "Cell sensitivity assays: the MTT assay." *National Center for Biotechnology Information*, doi:10.1007/978-1-61779-080-5_20. Abstract.
17. Ehrlick, M.E. et al. (2016) Rapid and efficient generation of oligodendrocytes from human induced pluripotent stem cells using transcription factors. PNAS 10.1073/pnas.1614412114
18. Barnes, J. Matthew, et al. "Tissue mechanics regulate brain development, homeostasis and disease." *Journal of Cell Science*. *US National Library of Medicine National Institutes of Health*, doi:10.1242/jcs.191742.
19. *ImageJ*. Dec 2019 ed. *National Institutes of Health*, imagej.nih.gov/ij/.