Role of the Extracellular Matrix in Brain Plasticity in Context of Pain Chronification

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INTRODUCTION

- In 2016, CDC estimated that 50M people were suffering from chronic pain, and close to 20M adults had chronic pain which was identified as “high impact chronic pain” [3].
- Pain chronification is often associated with memory deficits and cognitive impairment. This focuses on the hippocampus since it plays an essential role in memory formation and storage [1].
- There is currently no solidified source and function of chronic pain, but it is generally believed that neuronal plasticity and inflammation are either the source or significant contributors [4].

SIGNIFICANCE

- Understanding how microglia change when in contact with the extracellular matrix can help to better comprehend the evolvement of pain-related brain plasticity. This could benefit the 20% of the population that suffers from chronic pain by creating mechanism-based therapies for them [2].
- The extracellular matrix component of chronic pain is often overlooked in studies; however, it can contribute to the validity of the pain centralization theory.
- Currentl… treatable, not curable

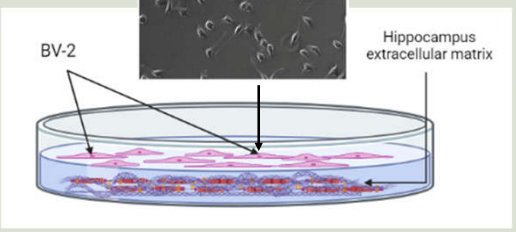

Figure 1: Depicts the culturing of BV-2 cells on a decellularized hippocampus collected from mice. Also included is an image of the morphology of BV2 cells under a Keyence BZ-X810.

MATERIALS & METHODS

Cell Culture: BV-2 cells derived from murine neonatal microglia are used as substitutes for primary microglia. All cells were plated in collagen-coated plates, except controls and were incubated for 1 hour prior to the experiments. After the incubation period, the plates were washed twice with 1X Phosphate buffer saline (PBS), followed by adding growth medium, supplemented with 10% FBS, 1% penicillin and streptomycin to avoid dehydration (DMEM +/+). Cells were seeded at 2.5 x 10^5 cells per well in 6-well collagen-coated plates plus media and were allowed to adhere and grow at 37°C with 95% humidity and 5% CO_2 to reach 80-90% confluence for 48 hours.

Cell Death Assay: After 48 hours of incubation, the supernatant of each well was collected and labeled. The wells were then washed with 1XPBS along with adding trypsin and centrifuged for 5 minutes at 3000 RPM. The tubes were decanted, and the pellets were resuspended in 200 μl of DMEM +/+. 20 μl of each sample tube was mixed with 20 μl of 4% trypan blue and were kept on ice for 5 minutes prior to analysis. Using a hemocytometer, the live cells and dead cells were distinguished, depending on the dye absorption, and were counted.

Morphology Assay: After 48 hours of incubation, the wells were washed with 1XPBS to remove any dead cells. The cells were then fixed using 1.5 ml of 4% formaldehyde for 11 minutes. After fixation, the 4% formaldehyde was discarded, and the cells were washed with 1XPBS for 5 minutes (3 times). After imaging the wells at 20X using Keyence BZ-X810, the cells were analyzed using Fiji (Image J-Win 32) software. Using polygon selection, each individual cell was traced and measured for its area and circularity.

Phagocytosis Assay: After 48 hours of incubation, 2 μm carboxylate-modified microspheres (F8827, Thermofisher) were added to the wells at the concentration of 5 beads per cell. The cells were then incubated at 37°C for 120 minutes. After incubation, the wells were then fixed using 1.5 ml of 4% formaldehyde for 11 minutes and were washed with 1XPBS. After imaging at 20X, the number of beads phagocytosed by each cell was counted and recorded.

RESULTS

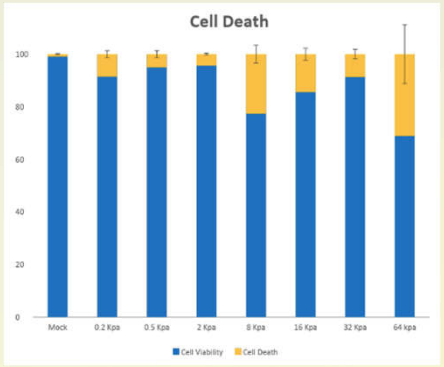

Figure 2: The various matrix Kpa values were compared to the mock for the percentage of cells that lived and died.

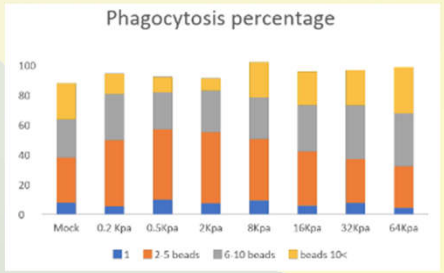

Figure 3: The percentage of bead groupings found in each of the various matrices.

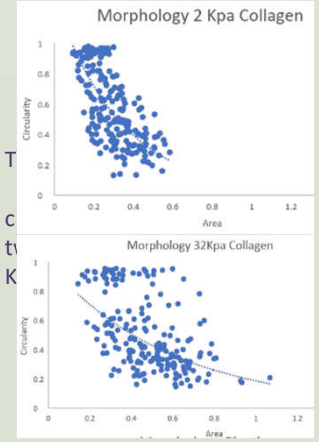

Figure 4: area and cells for different values.

CONCLUSION

- Cell death was the lowest at 2 Kpa which suggests that as being the optimal ECM rigidity for microglial growth
- The highest cellular complexity occurs at 2 Kpa
- As the rigidity increases, the area that microglia occupy also increases
- At 2 Kpa, microglia uptake 2-5 beads and as the rigidity increases, they phagocytose more
- ECM rigidity does have an effect on microglial morphology and function
- As the ECM rigidity increase, so does microglial activity

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