NRG1 Delivery via Sulfated-Hyaluronic Acid Microgels as a Neuroprotective Agent to Oxygen-Glucose Deprived Neuronal Cells

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Background

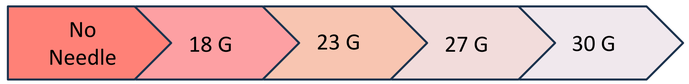
- Traumatic brain injury (TBI) remains a primary cause of death and disability worldwide with approximately 1.7 million cases annually in the U.S. alone [1]
- Ischemic neuronal apoptosis commonly occurs as a secondary injury of TBI [2]
- Previous research has reported the ability of neuregulin-1 (NRG1) to prevent extensive ischemic cortical damage in stroke models when administered prior to insult [3]
- Fewer studies have investigated the use of NRG1 as a treatment following TBI

Objective

- Observe the extent to which NRG1 improves the survival of oxygen-glucose deprived cells
- Validate that neuronal cells can be sufficiently stressed via oxygen-glucose deprivation (OGD) to properly mimic ischemic conditions
- Synthesize and characterize sulfated-HA microgels for future release studies

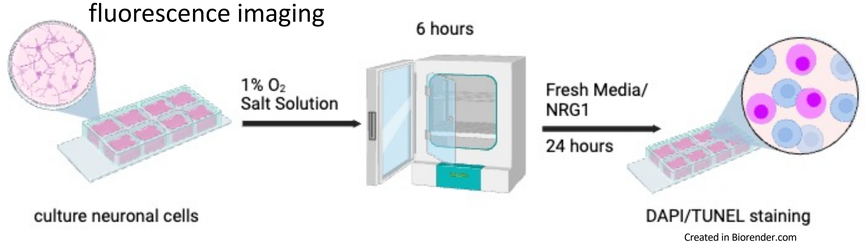
Sulfated HA Microgel Fabrication

- Bulk hydrogels were fabricated with sulfated hyaluronic acid (HA) and NRG1 inside of a syringe using via photo crosslinking with a UV lamp
- The bulk hydrogel was then fragmented by extrusion through multiple syringes, progressively extruding through smaller needle gauges

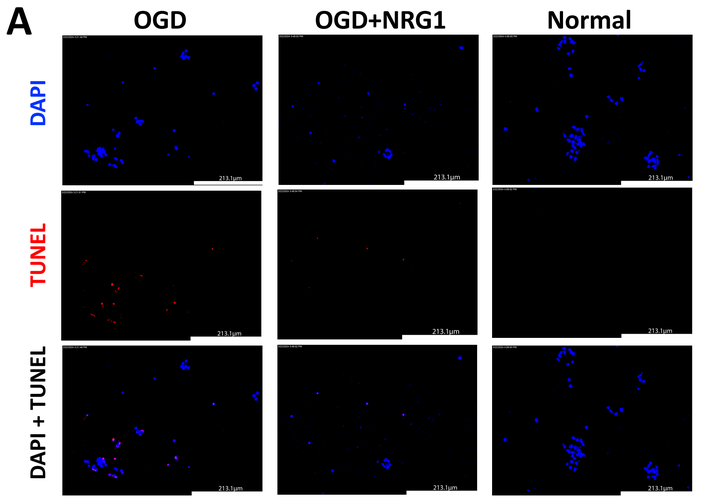


In vitro Bioactivity Assay

- E18 primary neuronal cells were dissociated from hippocampal tissue and culture on 8 chamber slides and incubated for 7 days
- OGD treatment groups were then treated with a glucose free Earle's balanced salt solution and placed in a hypoxia chamber for 6 hours (1% O_2)
- Salt solution was then replaced with fresh media/NRG1 and incubated for 24 hours
- Cell apoptosis was then observed via TUNEL staining and fluorescence imaging



Results



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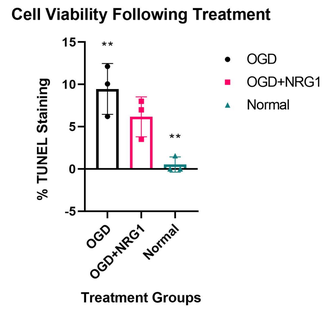

A) Fluorescence images showing all cells via DAPI and apoptotic cells via TUNEL staining for OGD, OGD+NRG1, and normally treated cells.

B) Percent average of apoptotic cells per chamber for OGD, OGD+NRG1, and normally treated cells were calculated and compared. There is a statistically significant increase in apoptotic cells between OGD and normally treated cells (**)

Conclusions

Neuronal cells were successfully stressed to mimic ischemic conditions through OGD however the NRG1 treatment did not have a significant effect on the number of apoptotic cells after OGD treatment. Cells appeared to cluster together prior to OGD potentially due to stress caused by poly-d-lysine (PDL) coating when attaching cells.

Future Work

- Replace PDL coating with a PDL/Laminin solution to help reduce stress on the cells during attachment
- Study the controlled release of NRG1 through the microgels
- Further characterization of a composite HA hydrogel system for neuroregeneration and drug delivery

Acknowledgments

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References

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