Determining the Efficacy of Adipose Stem Cell Derived Extracellular Vesicles As Preemptive Treatments for Ischemic Stroke

Fernando Carrillo[1,2*], Colin Esmonde[1,2] and Yan Li[1,2]

[2]Chemical & Biomedical Engineering, FAMU-FSU College of Engineering

FAMU-FSU College of Engineering — Chemical & Biomedical Engineering

Introduction

Stroke is a disease that many do not survive, and many who do suffer lifelong impairments with suffering quality of life. It is a disease that can strike without warning and may take as little as minutes to cause irreparable damage to the central nervous system. Currently, the only preventative and preemptive treatments for stroke consist of a variety of prescription medications that are associated with several potentially detrimental side effects. Adipose stem cell-derived extracellular vesicles (ASC-dEVs) are being considered as an alternative because they retain the regulatory functions of ASCs without the risks associated with ASC stem cell therapy. Due to having a relatively large size, ASCs have the potential to cause strokes by creating blockages in blood vessels; comparatively, their EVs are significantly smaller, effectively negating this risk. Additionally, EVs cannot self-replicate, and thus, cannot become cancerous. The goal of this research is to provide a viable alternative to prescription medication for those with elevated risk of stroke.

Cell culture/EV Isolation

ASCs are cultured in media comprise of α-MEM (minimum essential medium), FBS (fetal bovine serum), Pen-Strep (Penicillin-Streptomycin), and Sodium Bicarbonate which possess growth medium and antibiotic properties. The cells are incubated in standard body conditions of 37 degrees Celsius and about 5% carbon dioxide to mimic *in vivo* conditions. To isolate the EVs for use during experiments, the ASCs are cultured in EV-depleted media, and an Extra PEG approach is used for EV isolation [1] and nanoparticle tracking analysis (NTA) is used for quantifying the EVs.

Hydrogel Synthesis/EV Release Tracking

The hydrogels used during stroke modelling are 2 mg/mL collagen-based gels that have been preloaded with the varying EV doses. The gels are allowed to set and wash in media for 24 hours to prepare them for cell seeding. The loaded EVs are then tracked as they release for 6, 12, and 18 hours, respectively. To characterize and quantify the EVs, ELISA assays are done to check for the HSPA-8, CD81, and calnexin surface markers.

Works in Progress:

The experimental setup will consist of multiple groups all with EV and glucose depleted media to successfully mimic an ischemic stroke. A control group with no EVs loaded in the gel, and 3 experimental groups with varying doses of 1*10^9 EVs, 1*10^10 EVs, and a dose of 1*10^11 EVs loaded into gels will be used to test the effectiveness.

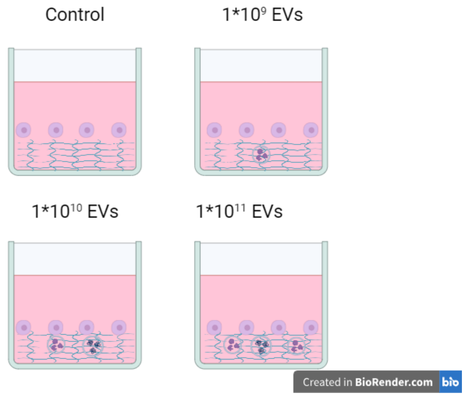

Figure 1: Experimental setup to be used during the oxygen-glucose deprivation (OGD) modeling created using BioRender [2].

For OGD modeling, each experimental group will be kept in a hypoxic chamber at 5% CO2, 92% N2, and 3% O2 to mimic the conditions experienced by neural cells during ischemia.

Tracking the release rate of the EVs from the hydrogels over the course of the OGD modelling, separate plates consisting of only the EV doses in the gels are incubated. Enzyme-linked immunosorbent assays (ELISA) are used to quantify the amount of EV that has diffused out the of the gels using the mechanism shown in figure 2.

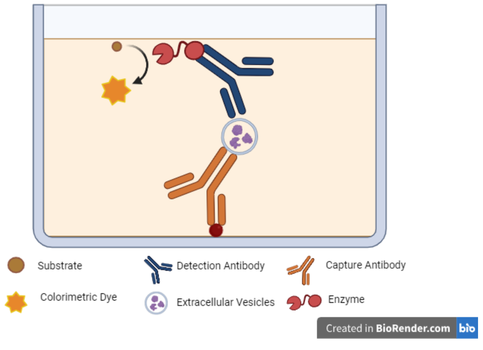

Figure 2: Basic composition and mechanism of ELISA assays created in BioRender.

The light intensity is measured using a colorimetry and used to determine EV concentration in the solution.

Results:

NTA was used to track the release of EVs following the cell culturing and EV Isolation procedures. Figure 3 shows the distribution of EVs by their size, and table 1 shows the concentration of the EVs that were obtained.

Average EV Concentration vs. EV Size

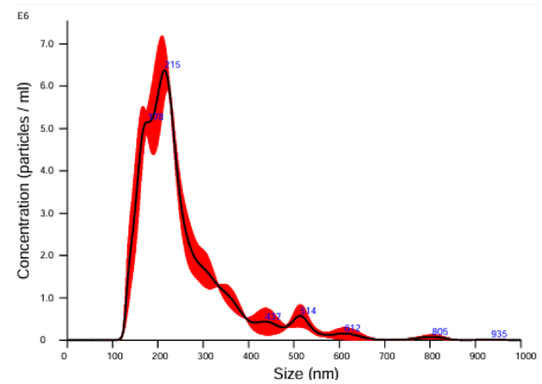

Figure 3: Average concentration of EVs relative to their size in nm

Concentration (EVs/ml)	Mean EV Size (nm)	Mode (nm)
8.61*10^8	255.5 +/- 3.7	193.8 +/-15.4

Table 1: Nanoparticle Tracking Analysis results.

Future Works:

Once all trials have been completed, cell viability tests will be run to determine the efficacy of the varying doses when subjected to varying lengths of stroke.

For statistical analysis, a two-tailed ANOVA test will be used to check for statistically significant differences in the results of the 4 different groups when subjected to the 3 different lengths of OGD modelling time.

Acknowledgements

Research funding provided by NIH (R01-NS125016 to YL).

References

[1] M. A. Rider, S. N. Hurwitz, and D. G. Meckes, Sci Rep, Apr. 2016, doi: 10.1038/srep23978.

[2] Created with BioRender.com