In vitro Characterization of Adipose Mesenchymal Stem Cells derived Extracellular Vesicles for Stroke Treatment

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Introduction

Resulting from interrupted blood supply, cerebral ischemic stroke is a leading cause of disability and mortality globally. At present, stroke treatments are limited to the intravenous injection of tissue-type plasminogen activator (tPA) and surgical embolectomy within a limited time frame, requiring alternative treatments. Mesenchymal stem cells (MSC) offer hope by promoting recovery through exosome secretion. This study focuses on *in vitro* characterization of extracellular vesicles derived from Adipose-derived MSC (ASC-EV) for stroke treatment.

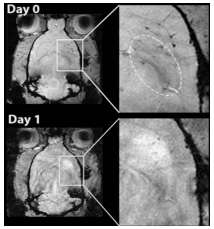

Figure 1: Gradient recalled echo MR image of a female rat with an ischemic lesion treated with 3D-cultured MSC-EV.

Cell culture/*in vitro* characterization

ASC are expanded in 2D culture using α-MEM (minimum essential medium) as growth medium. Cells are expanded using standard culture conditions to passage 7, characterized using Bradford protein assay, Western blot, and proteomics. Ultimately, cells are cultured to generate EV. An ExtraPEG approach is used for EV isolation [2].

Bradford Assay

The Bradford assay allows for the protein concentration present in ASC and ASC EV to be shown based on the shift in absorbance of Coomassie Blue. A microplate reader determines absorbances of reference samples with various protein concentrations to determine the amount of protein in an unknown sample.

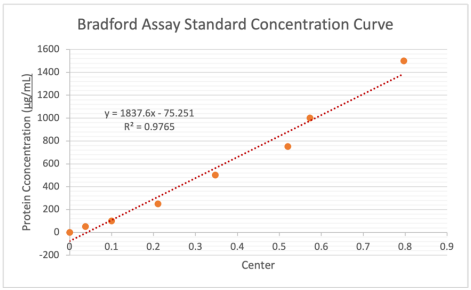

Figure 2: *The standard concentration curve shows protein concentration based on absorbance. The ASC EV protein concentration is 589.96 µg/mL while the ASC protein concentration is 828.848 µg/mL.*

Western Blot Procedure:

Western blots will be used to identify surface markers present in ASC and ASC EV to show successful EV isolation. Western blots utilize protein concentration information gained from Bradford assays to determine the amount of sample mixed with Laemmli buffer allotted into into each well. Electrophoresis is run and then the gel is removed from the cassette. The gel is encased in the blotting sandwich and ran once again. The gel will be blocked and primary and secondary antibodies will be introduced to the gel. The gel can be imaged to see the presence of surface markers in respective samples.

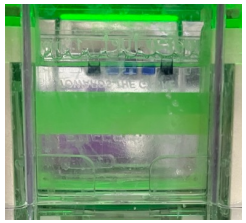

Figure 3: *Protein separation occurring via gel electrophoresis for Western blot. Wells loaded from left to right: sample ladder, lysed 2D ASC EV, lysed ASC, 3D ASC EV, sample ladder.*

Results:

Nanoparticle tracking analysis is completed using a NanoSight LM10-HS instrument to gain particle size distribution for P7 ASC EV.

Table 1: *Nanoparticle Tracking Analysis compares particle size for each sample of ASC EV against a PBS control.*

	Mean (nm)	Mode (nm)	Concentration (particles/mL)	Threshold Detection
ASC EV 1	191.3	180.7	4.14E+08	6
ASC EV 2	201.7	142.7	4.92E+08	6
ASC EV 3	187.8	153.5	4.37E+08	7
ASC EV 4	247.1	189.7	4.85E+08	7
ASC EV 5	218.6	145	3.55E+08	6
ASC EV 6	215.8	132.1	3.66E+08	6
PBS	183.5	179.5	2.50E+08	6

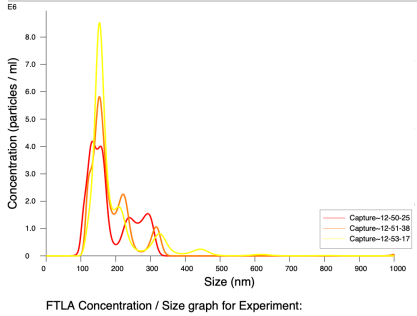

Figure 4: *The data profile determines the amount of noise found in the sample. For the data collected, each profile has some noise present.*

Future Works

Current aims are to show the presence of certain protein markers using Western Blotting. If EV isolation methods are successful, ASC EV will have markers for CD81 while ASC alone will have markers for Calnexin. Proteomics testing can be used to identify protein content in ASC and ASC-EV to see if there are any shared proteins [3]. Proteomics can allow for the detection of proteins that are unique to EV. This will aid in proving why EV can act as an effective potential stroke treatment. Polymerase Chain Reaction (PCR) is a process that will show the levels of mRNA in ASC-EV samples [4]. PCR primers can be added individually to different samples of ASC EV to detect the presence of individual RNA strands in the sample.

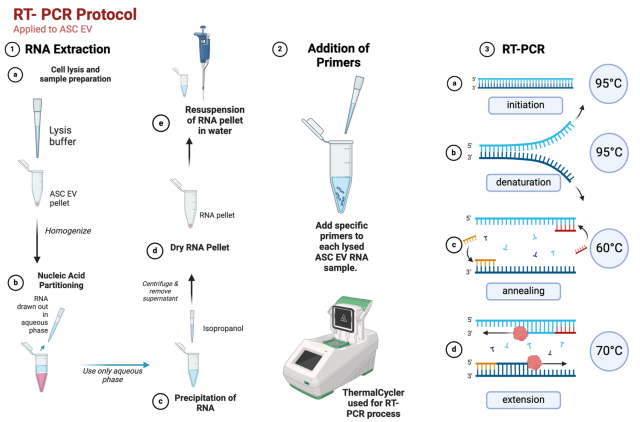

Figure 5: *BioRender schematic showing PCR preparation protocol for RNA extraction, adding primers, and the steps of PCR.*

Acknowledgements

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References

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