Exosomes of Placental Mesenchymal Stromal Cells: Paracrine Signaling and Therapeutic Potential

Danielle Wang[1], Tara Narasimhalu[1], Priyadarsini Kumar[1], Aijun Wang[1], Diana Farmer[1]

[1]Surgical Bioengineering Laboratory, Department of Surgery, University of California Davis, Sacramento, California

INTRODUCTION

Mesenchymal stromal cells, derived from the chorionic villi of human placenta (PMSCs), exhibit both multipotent differentiation potential and immunomodulatory capacity.[1] These characteristics make PMSCs likely to be utilized into therapies in the fields of stem cell transplantation and regenerative medicine. Traditionally, treatment of myelomeningocele (a type of spina bifida) has been through surgery after birth. However, recent studies have shown that *in-utero* repair of myelomeningocele has led to better clinical outcomes.[2] Further, the Surgical Bioengineering Lab at UC Davis has found that PMSCs seeded on extracellular matrix can potentially be used as a therapeutic agent for *in utero* repair.[3] This approach significantly improved motor function in ovine myelomeningocele.

PMSCs exert their function through paracrine secretion. Exosomes are one of the extracellular vesicles secreted from PMSCs. They contain biological material, such as protein, mRNA and microRNA. Exosomes are hypothesized to play a role in the intercellular communication of PMSCs. Characterizing and engineering PMSC-exosomes is essential to translating their therapeutic function to cell free therapy of clinical disorders.[4,5]

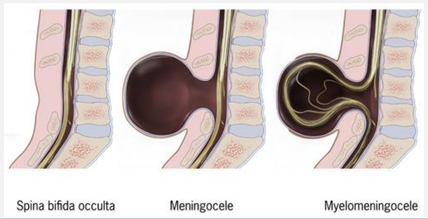

Figure 1: Anatomy of Myelomeningocele (CDC: Spina Bifida Basics)

OBJECTIVES

1. **Generate large quantities of exosomes.** Because exosomes are secreted in small quantities, we used established protocol to generate a large quantity of PMSC-exosomes.
2. **Characterize the generated exosomes.** In order to study the properties of exosomes, we characterized them using Western Blot Analysis, Nanoparticle Tracking Analysis, RNA analysis and Flow Cytometry.

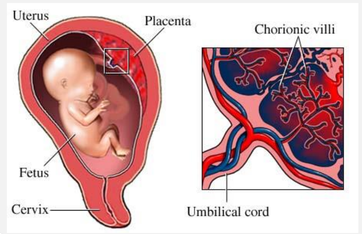

Figure 2: Placenta villus structure (Campbell Biology, 9th edition)

MATERIALS & METHODS

Isolation of Exosomes

We used the established protocol to generate a large quantity of native PMSC-exosomes. This was done by performing differential ultracentrifugation with stem cell culture in conditioned media. Three donor cell banks were used: 465 (Donor #1), 615 (Donor #2), 488 (Donor #3)

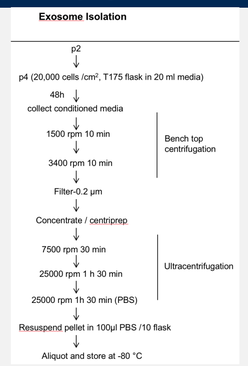

Figure 3: Exosome Isolation

Characterization of Exosomes

1. Western Blot
CD63 is a common cell surface protein used as marker for exosomes. Western blot analysis was run to determine if the isolate contains CD63 markers

2. Nanoparticle Tracking Analysis
In order to determine the size and concentration of exosomes, we performed Nanoparticle Tracking Analysis.

3 RNA analysis
The three lineages of PMSC-exosomes were sequenced to identify their specific RNA content and composition.

4. Exoflow
We performed selective exosome capture to identify Galectin-1 as a surface biomarker in PMSC-exosomes. This was done by incubating exosomes with avidin conjugated magnetic beads and conjugated biotin with Galectin-1. Avidin is a biotin binding protein. Lastly, we added a secondary antibody (Exo-FITC) as a fluorescent stain to visualize the complexes.

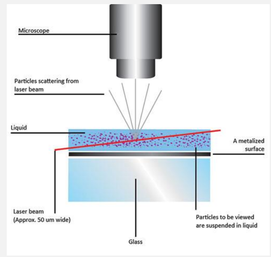

Figure 4: Nanoparticle Tracking Analysis (Malvern Panalytical)

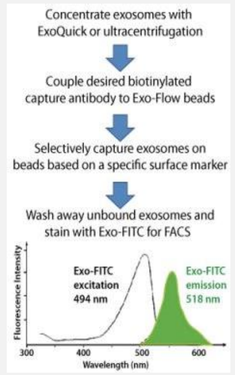

Figure 5: Exo-flow protocol (BioCat GmbH)

RESULTS

Characterization Results

Figure 6: Western blot analysis of exosome marker CD63 protein.

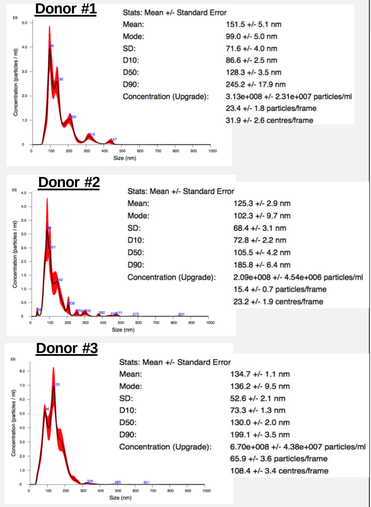

Figure 7: Characterization of exosomes by Nanoparticle Tracking Analysis (NTA) from 3 donor cell banks

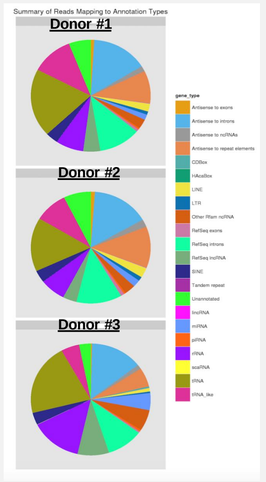

Figure 8: RNA composition of exosomes

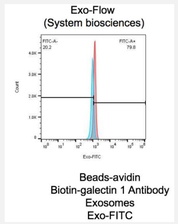

Figure 9: Galectin-1 flow cytometry.
- Blue=Negative
- Red=Positive. **86% exosomes are positive for Galectin-1 on surface.**

CONCLUSIONS

We have isolated and characterized PMSC-derived exosomes by differential ultra centrifugation.

- They express **CD63 protein**, a classic exosome marker.
- Our data from NTA shows that exosomes from each of the 3 donor cell banks have measurable sizes with a **mean of 137.2 nm** and a **mode of 112.5 nm**. Expected exosome size range is 50-150 nm.
- Next generation RNAseq analysis of exosomes showed presence of several RNAs; **tRNA and antisense to introns make up the highest composition in all three donors**. However, each of the three donor cell banks has different compositions, which indicates the possibility of donor to donor variability.
- Flow cytometry revealed that a significant quantity of exosomes contain **Galectin-1** (a protein that imparts multiple cell signaling functions) present on the surface of the exosomes. This could potentially be used for surface modifications of exosomes for an effective targeted therapy.

Further research involves altering the composition of exosomes and characterization of other surface biomarkers that will augment their function. More research within the realm of bioengineering and *in vivo* experimentation is necessary to develop therapeutic treatments with PMSC-nanovesicle delivery.

REFERENCES

1. Abumaree MH, Al Jumah MA, Kalionis B, et al. Phenotypic and functional characterization of mesenchymal stem cells from chorionic villi of human term placenta. Stem Cell Rev 2013;9:16-31.
2. "UCSF Fetal Treatment Center." *Spina Bifida (Myelomeningocele) | UCSF Fetal Treatment Center*, fetus.ucsf.edu/spina-bifida.
3. Kabagambe S, Keller B, Becker J, et al. Placental mesenchymal stromal cells seeded on clinical grade extracellular matrix improve ambulation in ovine myelomeningocele. J Pediatr Surg 2017.
4. Jarmalaviciute A, Pivoriunas A. Exosomes as a potential novel therapeutic tools against neurodegenerative diseases. Pharmacol Res 2016;113:816-22.
5. Zhang Y, Chopp M, Liu XS, et al. Exosomes Derived from Mesenchymal Stromal Cells Promote Axonal Growth of Cortical Neurons. Mol Neurobiol 2016.
5. Taylor, Martha R., et al. *Campbell biology: concepts & connections*. Pearson, 2010.

ACKNOWLEDGEMENTS

We would like to acknowledge the guidance of our research mentors at the UC Davis Surgical Bioengineering Laboratory: Dr. Aijun Wang, Dr. Diana Farmer, Dr. Priyadarsini Kumar, Dr. Kewa Gao, and Christopher Pivetti.

Additionally, we would like to express our gratitude to the Medical Student Research Fellowship program at the UC Davis School of Medicine and our donors. The Naomie K. King Fellowship Fund and the Morton Levitt Memorial Endowment Fund graciously supported Danielle Wang and Tara Narasimhalu, respectively.