Placental Mesenchymal Stem Cells and Extracellular Vesicles on an Extracellular Matrix Improved Motor Function Recovery After Acute Spinal Cord Injury

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Introduction

Spinal cord injury (SCI) is a devasting disease with no effective cure. We have shown that placental mesenchymal stromal cells (PMSCs) applied *in utero* improve ambulatory function in an ovine model of spina bifida and have begun the first-in-human clinical trial for fetal spina bifida. PMSCs and extracellular vesicles (EVs) have neuroprotective properties and we hypothesized that PMSCs and PMSC-EVs would provide a similar neuroprotective effect in SCI.

Figure 1. Baby, Robbie: First patient in the world to receive stem cell therapy for In Utero Repair of Myelomeningocele *(Tomiyoshi, 2022)*

Methods

PMSCs were expanded in a serum-free media. Flow cytometry, nanoparticle tracking analysis, neuroprotection assays were used to characterize PMSCs and PMSC-EVs. The *in vivo* studies included injured and uninjured rats. The injured rats underwent a laminectomy at C5, followed by a unilateral spinal cord injury (SCI). The uninjured rats underwent a laminectomy at C5, but did not sustain an SCI.

The injured groups were treated with either:

- *extracellular matrix (ECM) patch alone* or
- *ECM patch with PMSCs* or
- *ECM patch with PMSC-EVs*

The rats were sacrificed at 8 weeks post injury, after motor testing was complete, for histology.

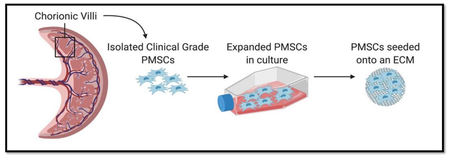

Figure 2: The extraction and expansion of PMSCs

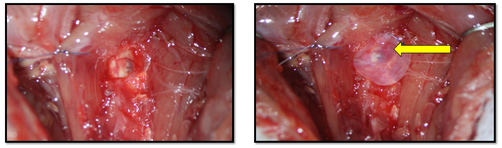

Figure 3: Spinal cord injury repair at C5 vertebra with the arrow pointing towards a PMSC patch

Results

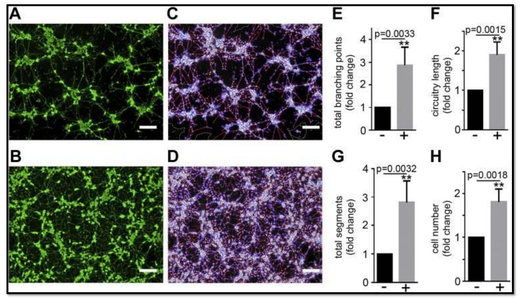

Figure 4: A-D. Neuroprotective Assay conducted with staurosporine-treated SH-SY5Y cells shows increased neurite growths in the presence of PMSCs (B and D). E-H: adding PMSCs to the cells resulted increased total branching points (E), increased circuitry length (F), increased total segments (G) and increased cell number (H). *(Kumar, 2019)*

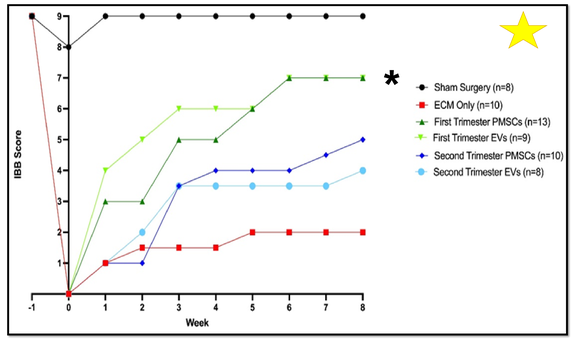

Figure 5: Motor data taken over 8 weeks, using the IBB forelimb recovery scoring scale. Higher scores correlate with better performance.

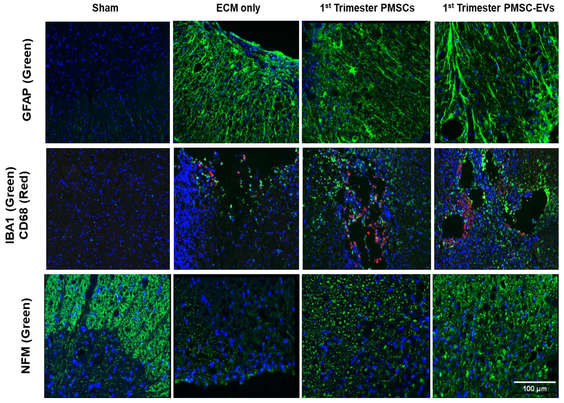

Figure 6: Representative Immunofluorescent Images for Sham, ECM only, 1st Trimester PMSCs and 1st Trimester PMSC-EVs. The stains included are GFAP for astrocytes, IBA1 for microglia, CD68 for peripheral macrophages and neurofilament M (NFM) for axons.

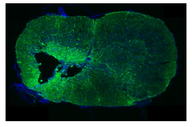

Figure 8: Whole injured spinal cord for reference.

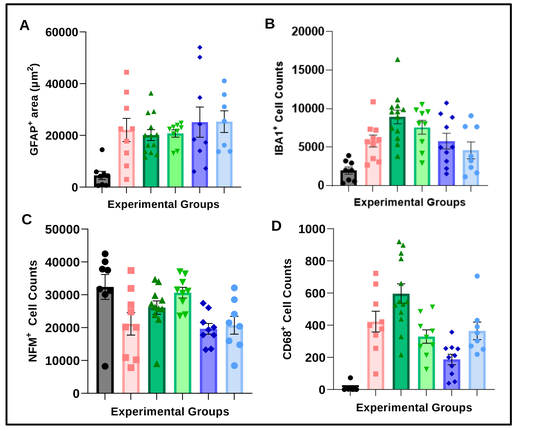

Figure 7: A) The expression of astrocytes measured by GFAP B) The microglia measured by IBA1 C) The axon counts measured by NFM D) Peripheral macrophage recruitment measured by CD68

Conclusions

1. PMSCs and PMSC-EVs significantly improved motor function recovery in a rodent model of SCI
2. 1st Trimester cells performed better than 2nd trimester cells
3. 1st trimester PMSCs and 1st trimester PMSC-EVs follow similar trends and 2nd trimester PMSCs and PMSC-EVs do the same
4. PMSC-EVs are a novel, cell-free therapeutic
5. PMSCs and PMSC-EVs increase neuron counts, microglia involvement and peripheral macrophages in spinal cord injury
6. Astrocytes need to be further studied

Further Studies

- Modifying PMSCs and PMSC-EVs for specific targeting to neurons and glial cells; loading PMSC-EVs with cargo
- Testing PMSCs and PMSC-EVs with spinal cord injuries in bigger animals such as sheep
- Narrowing the focus to 1st trimester PMSCs and PMSC-EVs only
- Using PMSCs and PMSC-EVs for the treatment of other CNS injuries such as traumatic brain injury

Citations

- Tomiyoshi, T. *World's first stem cell treatment for spina bifida delivered during fetal surgery*. https://health.ucdavis.edu/news/headlines/worlds-first-stem-cell-treatment-for-spina-bifida-delivered-during-fetal-surgery--/2022/10. December, 2022
- Kumar P, et al. Neuroprotective effect of placenta-derived mesenchymal stromal cells: role of exosomes. *FASEB J*. 2019

Acknowledgements

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