Bioengineered SMaRT Human Neural Stem Cells for Scar Degradation and Functional Recovery in a Chronic Spinal Cord Injury Model

Nayaab Punjani[A,C], Candidate, Hons. BSc., Christopher S. Ahuja[B,C], MD, Michael G. Fehlings[B,C], MD, PhD, FRCSC, FACS

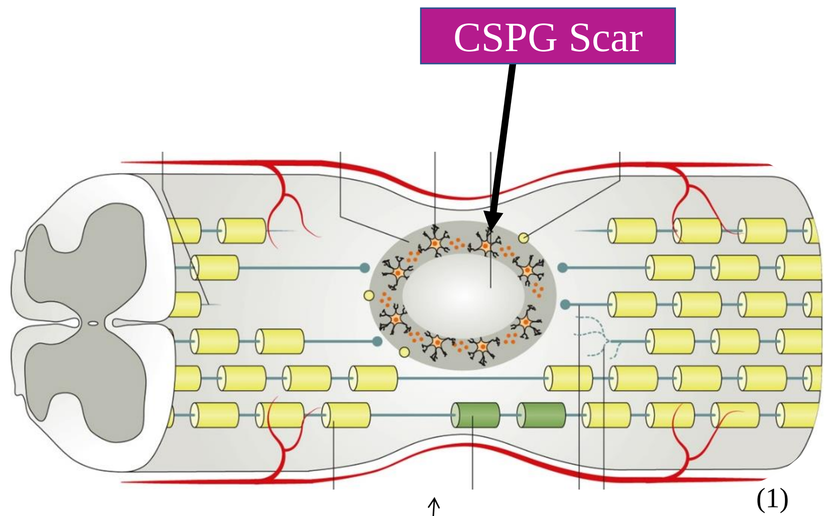
[A] University of Toronto Scarborough, [B] Department of Surgery [University of Toronto], [C] Genetics and Development [Krembil Research Institute]

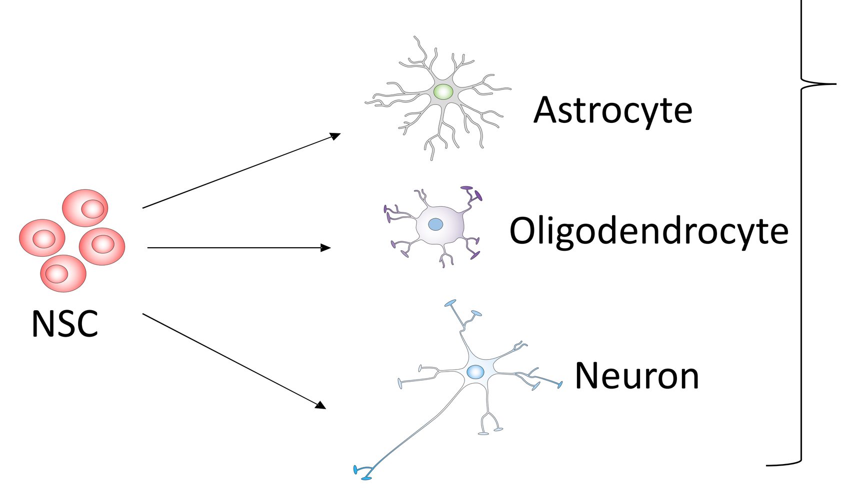
Background: Spinal Cord Injury

Spinal cord injury (SCI) leads to a loss of productivity and daily functional ability.

The cost of care for patients can range from $1.1-4.6 million US over the course of their lifetime[1].

At the chronic stage (> 6 months after injury), an inhibitory scar forms from the aggregation of proteins called chondroitin sulfate proteoglycans (CSPGs)[1]. This prevents all cells of the central nervous system (CNS) from regenerating, thus preventing recovery.

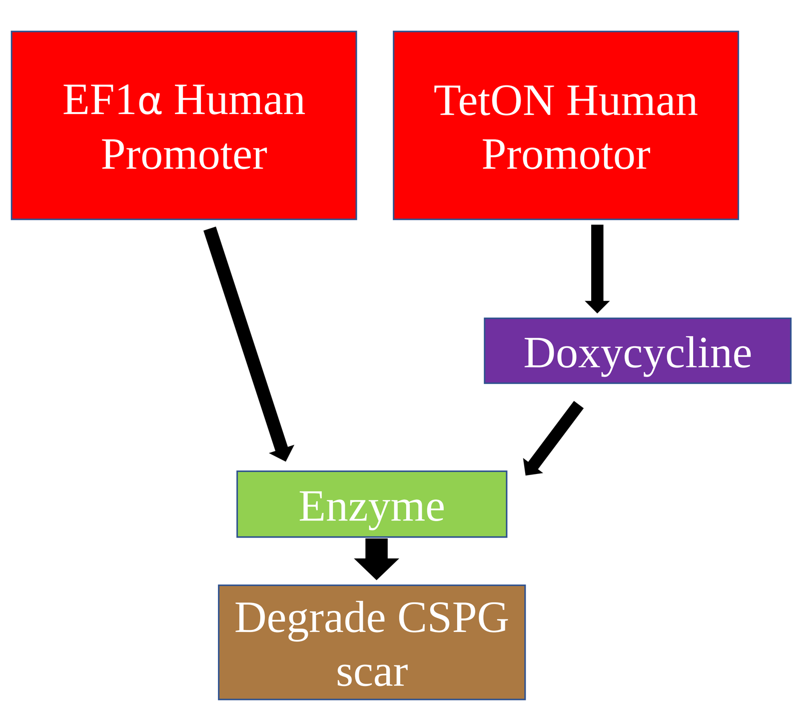




Human induced pluripotent stem cell-derived neural stem cells (hiPS-NSCs) can differentiate into different cell types needed by the central nervous system (CNS)[2], but they cannot get past the inhibitory scar.

SMaRT Cells[3]

Spinal **M**icroenvironment Modifying **a**nd **R**egenerative **T**herapeutic (SMaRT) cells are a genetically-engineered line of hiPS-NSCs that lead to targeted release of CSPG-degrading enzymes to help break down the scar.



2 forms of the SMaRT cell:

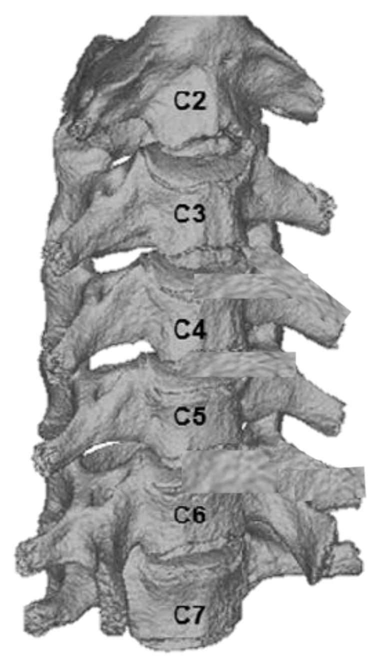
- One involves the EF1α promoter for continuous release of CSPG-degrading enzymes.
  - Focus of this poster
- The other involves the TetON promoter[4], which requires the antibiotic doxycycline to control the release of enzymes.
  - Study still in development

Hypothesis

The enzymes released by SMaRT cells will be able to degrade the inhibitory CSPG scar and lead to tissue repair by differentiating into various cell subtypes, thus providing a treatment for chronic SCI.

Methods

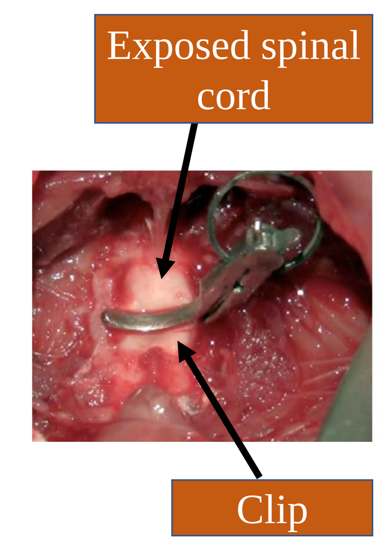
Rat Model



Cervical spine of rats has 7 bones C1-C7 [5]

The most common injury in humans is at the cervical region of the spinal cord (highest portion) [1].

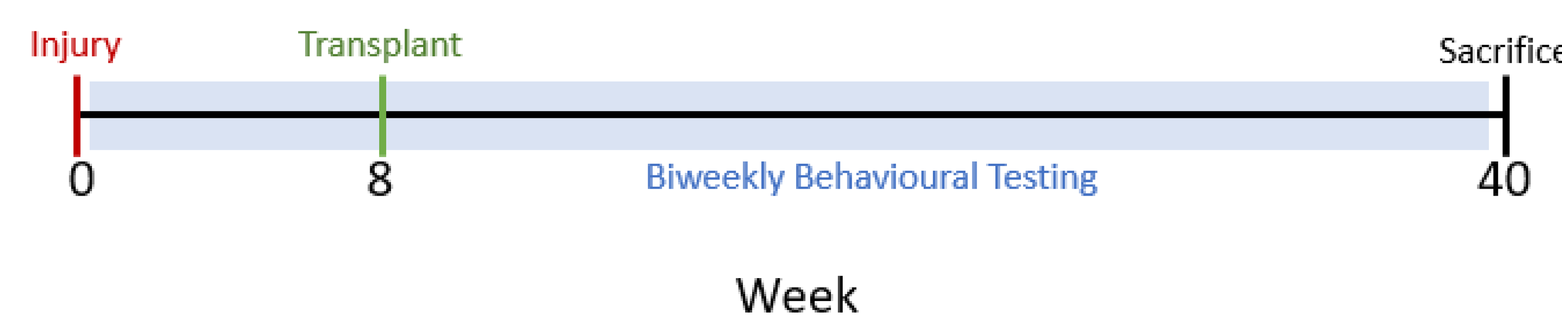
The rat model involves the use of a clip contusion injury at the C6-7 portion of the spine, which is a well-established model of traumatic cervical SCI.



Study Timeline

Immunodeficient rats (N=60) at the chronic stage of SCI were randomized into one of the following conditions:

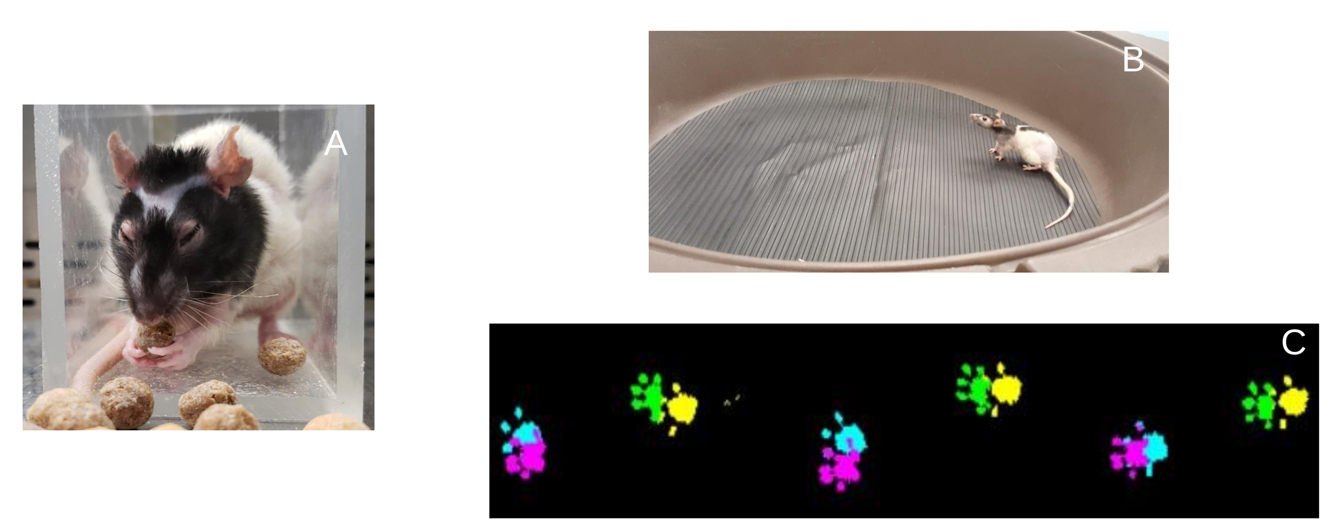
(1) NSCs
(2) SMaRT enzyme-expressing NSCs
(3) Vehicle control
(4) Sham (uninjured) control



Behavioural Testing

Behavioural tests were used to assess sensorimotor functional recovery of different aspects:

- Forelimb: IBB (fine movements; A), Grip Strength
- Locomotion: BBB (open-field locomotion; B), CatWalk Gait Analysis (C)

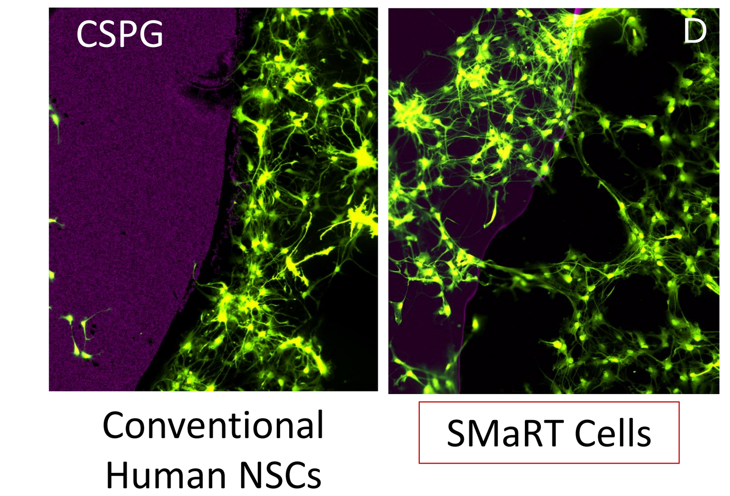


Data collection and analysis of results from behavioural testing is ongoing to determine the degree of functional recovery resulting from these treatments.

Results

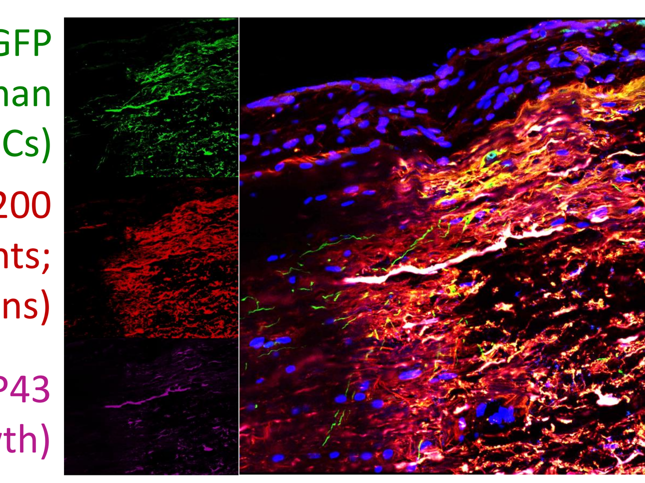
In vitro – Cell Analysis

CSPG spot assay - SMaRT cells are able to break down CSPG and grow within the CSPG region compared to conventional NSC (D)



Immunohistochemistry (IHC) – Tissue Analysis

Mature neuronal markers and growth seen at 40-weeks post-injury (E)



Conclusions and Future Directions

- SMaRT cells show promise as a potential treatment for spinal cord injury based on the *in vitro* and tissue analysis conducted, however the current *in vivo* results have yet to be fully analyzed to determine the degree of axonal growth, functional recovery, and scar degradation.
- IHC analysis shows that neurons differentiated from SMaRT cells show growth even at 40-weeks post injury, thus demonstrating its potential capacity for cellular regeneration and tissue repair at the chronic SCI stage.
- A new version of the SMaRT cell is currently being assessed in a 22-week study using the TetON human promoter which allows for release of enzymes to be controlled by the intake of the antibiotic doxycycline.

References

1. Ahuja, C.S., Wilson, J.R., Nori, S., Kotter, M.R.N., Druschel, C., Curt, A., & Fehlings, M.G. (2017). Traumatic spinal cord injury. *Nature Reviews| Disease Primers, 3:17018*, 1-21. doi:10.1038/nrdp.2017.18
2. Khazaei, M., Ahuja, C.S., & Fehlings, M.G. (2017). Induced Pluripotent Stem Cells for Traumatic Spinal Cord Injury. *Frontiers in Cell and Developmental Biology, 4:152*, 1-9. doi:10.3389/fcell.2016.00152
3. Ahuja, C.S., Khazaei, M., Baig, S., Jainetri, M., Chan, P., & Fehlings, M.G. (2017). SMaRT Human Neural Stem Cells to Modify Scar and Optimize Regeneration After Traumatic Cervical Spinal Cord Injury [Abstract]. *Journal of Neurotrauma, 34*(13), A-2. https://doi.org/10.1089/neu.2017.29011.abstracts
4. Addgene (n.d.) *Tetracycline (Tet) Inducible Expression.* www.addgene.org/collections/tetracycline/#on.
5. Jaumard, N., Leung, J.M., Gokhale, A.J., Guarino, B.B., Welch, W.C., & Winkelstein, B.A. (2015). Relevant Anatomic and Morphological Measurements of the Rat Spine: Considerations for Rodent Models of Human Spine Trauma. *Spine, 40 20*, E1084-92 .
6. Charles River (n.d.). *RNU Rat*. https://www.criver.com/products-services/find-model/rnu-rat?region=3611

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