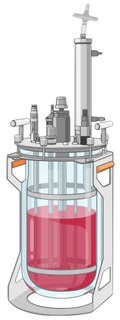
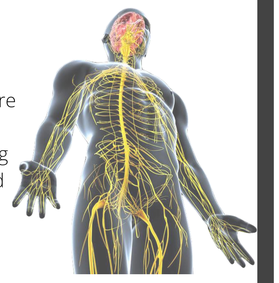


Bioprocess Pilot for Large Scale Production of Human Schwann Cells

Sanchit Chopra[1], Breanna Borys[1, 3], Erin Roberts[1, 3], Tak Ho Chu[4], Rajiv Midha[4], Michael Kallos[1, 2, 3]

[1]Pharmaceutical Production Research Facility, [2]Department of Chemical and Petroleum Engineering, Schulich School of Engineering, [3]Biomedical Engineering Graduate Program, [4]Department of Clinical Neurosciences, University of Calgary, 2500 University Drive NW, Calgary, AB, CANADA T2N 1N4

Introduction



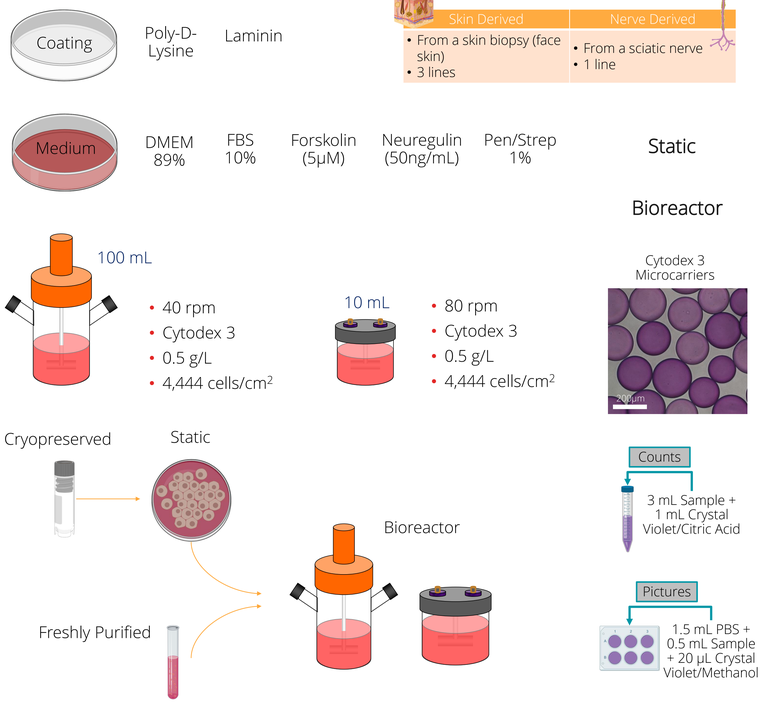
Severe peripheral nerve (PN) injury affects 2.8% of trauma patients treated every year in Canada. Current treatments are suboptimal with only 25% of patients recovering full motor function and only 3% recovering full sensory function leading to life-long functional impairment. It has been demonstrated that Schwann cells (SCs) aid in the regeneration of axons in the PN system by myelinating axons and producing extracellular matrix components to restore function.

Pre-clinical trials of using SCs for PN injuries have shown promising results but there is still a gap in a robust and reproducible method of producing enough cells for clinical scale. Bioreactor based bioprocesses offer significant advantages for efficient expansion for the purpose of cellular therapies.

Objectives

The aim of this project was to pilot a bioprocess for the expansion of human-SCs using bioreactors for the development of an autologous therapy.

Materials/Methods



Results

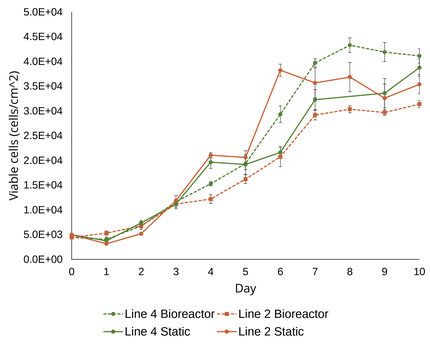


Figure 1. Human Schwann cell (SC) growth curves comparing 100 mL bioreactors vs static.

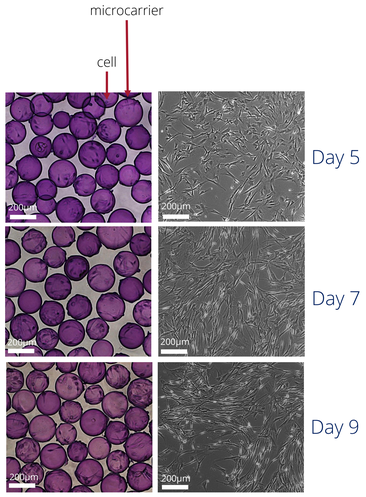


Figure 2. *Left:* Human SC attached on microcarriers. *Right:* Human SC grown on static dishes.

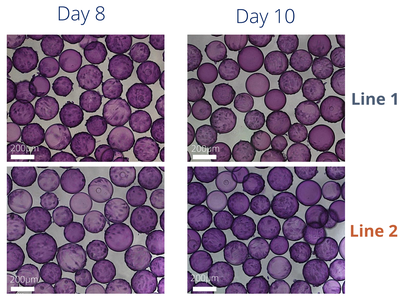


Figure 4. Human SC attached on microcarriers on days 8 and 10 in 10 mL spinner flasks

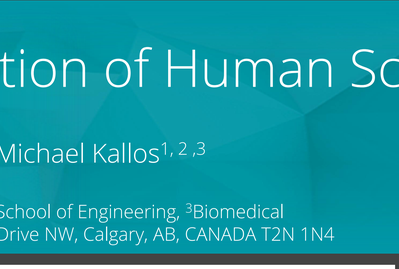


Figure 3. Human SC fold expansions on days 8 and 10 in 10 mL spinner flasks

~400,000 human-SC's isolated from human nerve/skin → inoculate 10 mL (x5) spinner flasks for expansion

Maximum Fold Expansion

	10 mL	Static
Line 1	18 on day 8	7 on day 8
Line 2	13 on day 10	8 on day 6
Line 3	16 on day 10	N/A
Average Fold Expansion	**16 in 8-10 days**	7.5 in 6-8 days

Figure 5. Summary of fold expansions of various lines grown in dynamic and static culture

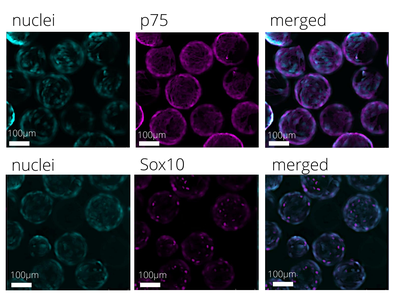


Figure 6. Confocal images of human-SCs grown on microcarriers stained with p75 and Sox10 SC markers

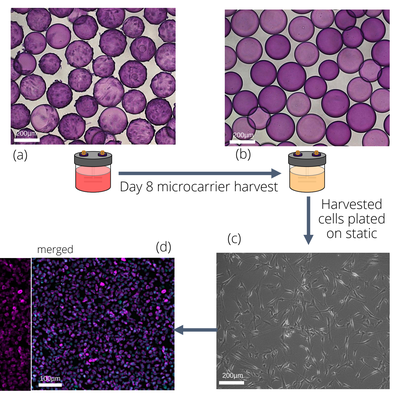


Figure 7. (a) Day 8 of human-SCs grown on microcarriers, (b) Empty microcarriers post harvest, (c) harvested cells plated on static, (d) confocal images of single cells stained with p75.

Discussions

The current methods of expanding human-SCs using static culture flasks are cost inefficient, labour intensive and may have variability between different culture vessels.

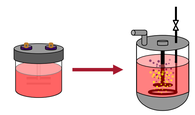
The use of bioreactors significantly increased Schwann cell expansion efficiency and was consistent between different human lines. On average, the growth of human-SC's in 10 mL spinner flasks achieved two times (2x) the fold expansion than that of static when compared across 3 different human lines.

In all cases, the human-SCs were able to maintain their phenotype by expression of p75 and Sox10.

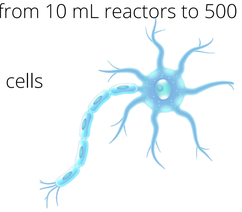
Optimizing harvesting protocols and developing a direct passage protocol from 10 mL reactors to 500 mL computer controlled reactors should be conducted to achieve higher cell densities needed for clinical trials.

This study shows promising applications of using stirred suspension bioreactors to expand human-SCs for future clinical applications for peripheral nerve injuries.

Future Directions



- Testing more human cell lines
- Optimizing harvesting protocols
- Develop protocols for passaging harvested cells from 10 mL reactors to 500 mL DasGip computer controlled reactors
- Analyzing regenerative capability of the Schwann cells
  - Myelination assays, unbiased proteomics
- Conduct tests in regard to release criteria
  - Sterility, purity, viability, endotoxin



References & Acknowledgements

Thank you to the Pharmaceutical Production Research Facility (PPRF) and our collaborators to make this project possible.

Contact: sanchit.chopra@ucalgary.ca
403 918 5633