



Promoting Oligodendrocyte Differentiation from Human Neural Stem Cells for 3D Myelination Models

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Introduction

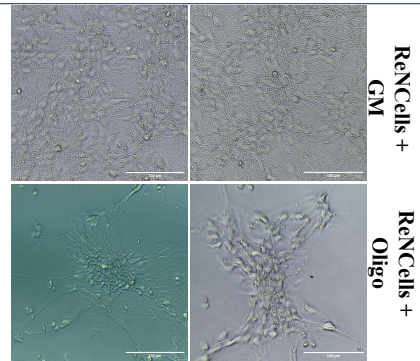
- Myelination by oligodendrocytes is essential for efficient neural signal transmission and overall central nervous system function. Disruptions in this process are implicated in a range of neurological disorders, yet studying myelination in controlled environments remains challenging due to the complex spatial and cellular organization of neural tissue.
- Hydrogels provide a unique opportunity to illustrate cell-to-cell interactions with spatial assembly compared to traditional 2D *in vitro* models. Novel gelatin microgel assemblies designed in Jeong Lab increase the nutrient transfer, cell spreading, and growth for these spatial models.
- In this work, we evaluate the effects of ScienCell™ Oligodendrocyte Growth Supplement of increasing oligodendrocyte lineage differentiation of ReNcell VM in the microporous hydrogel model to investigate neuron-oligodendrocyte interactions and support physiologically relevant myelination investigations.

Cell Viability

Cells cultured in oligodendrocyte media exhibited reduced viability and poor attachment, indicating suboptimal differentiation conditions. 2D cultures of ReNcell were maintained in GM or Oligo+ media for one week. Cells were seeded into a well plate and switched to DM for 3 days. Cells in the Oligo media exhibited marks of poor cell health and detached in large percentages compared to controls. Media conditions need to be optimized. Possible issues with current delivery include...

- Need for increased media changes
- Lack of appropriate growth factors for ReNcell culture
- Failure of the supplement to interact with serum free media

Figure 1:



Microgel Fabrication

Microgel Solution:
- Gelatin Solution solution: 10% wt gelatin solution dissolved in DI water

Microgel Fabrication:
- Emulsion is formed between the gelatin solution and olive oil at 55°C with a stir bar
- Solution is added dropwise to encourage dispersion
- After 30 minutes, the emulsion is placed on ice and chilled acetone is added
- The chilled emulsion is vacuum filtrated and then sterilized

Crosslinking and Seeding Protocol:
- Sterilized microgels are rehydrated with growth media supplemented with 20 ug/mL of laminin in a 48 well plate
- Post rehydration, microgels are seeded with neural stem cells and crosslinked using microbial transglutaminase

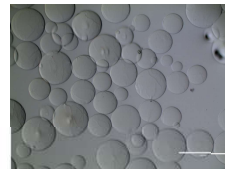
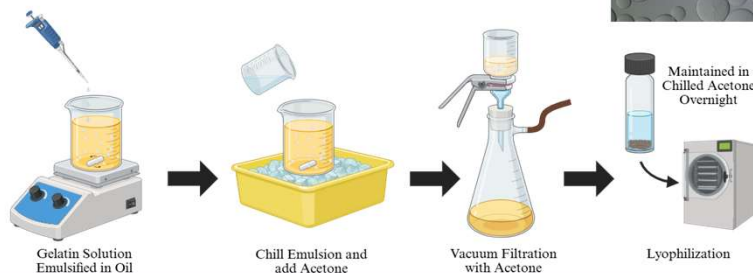
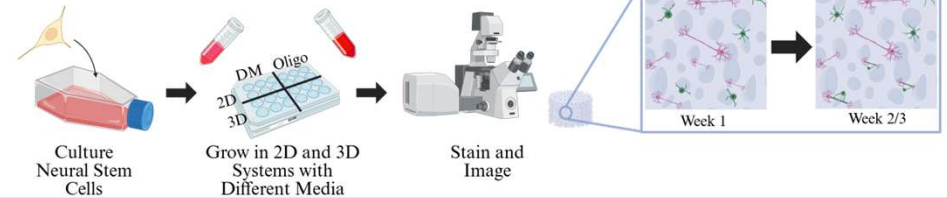


Figure 2: A B



Experimental Design

A stable neuronal stem cell line (ReNcell VM) are first expanded in culture with growth media (GM) and subsequently seeded into a multiwell platform (in 2D cultures or 3D hydrogel systems). To increase oligodendrocyte differentiation **oligodendrocyte supplement** is added to some samples. Samples are maintained under controlled conditions and analyzed using microscopy to assess cellular interactions and myelin formation over time. Representative imaging demonstrates progression from early network formation (Week 1) to increased oligodendrocyte engagement and myelination (Weeks 2-3).



Oligodendrocyte Differentiation in 2D vs 3D Systems

2D AND 3D samples were cultures for 2 weeks in DM or Oligo Media. The samples were stained for nuclei (blue), acton (red), and either oligo 2 or myelin basic protein (MBP) in green. There were no detectable amount of green in any condition indicating no noticeable oligodendrocyte development or myelin production. The Oligo media seemed to increase proliferation compared to the control differentiation media as seen by the abundance of nuclei and lack of actin development. This could be due to the presence of --- or ---- in the supplement, the two additive growth hormones used to keep ReNcell VM in a proliferation state and not a differentiation state.

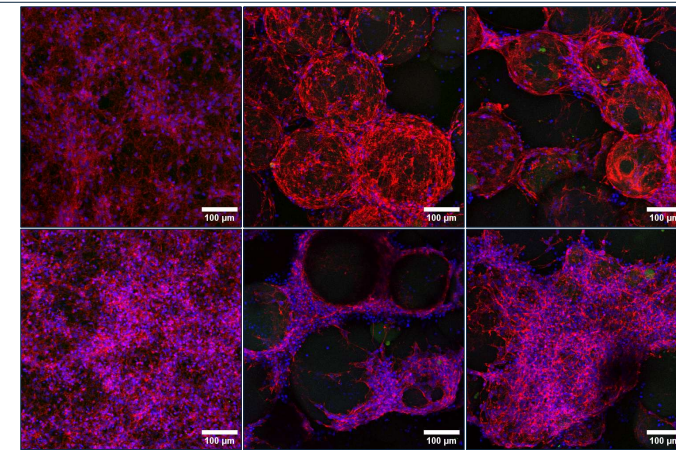


Figure 3

Conclusions and Future Directions

Conclusions

- Current differentiation conditions did not support effective oligodendrocyte maturation
- This study was unable to recapitulate the findings of Chen et al. of which this study was based
- The 2D and 3D systems exhibited...

Limitations

- Unknown media composition of ScienCell™ Oligodendrocyte Growth Supplement
- Short time windows when compared to known oligodendrocyte differentiation models
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Future Directions

- Optimize oligodendrocyte differentiation media
- Incorporate defined growth factors
- Reevaluate myelination using specific markers in 2D and 3D systems
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References

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