Peripheral Nerve Regeneration

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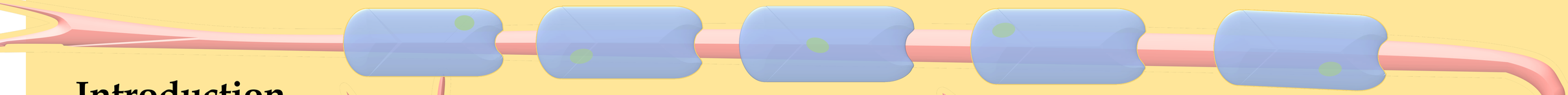

Introduction

- Peripheral Nerve Damage has limited treatment options and a low recovery rate
- Recovery rate is improved by supplementing treatment with Schwann cells (SCs)
- SCs harvest for treatment is limited.
- SCs can be derived from stem cells

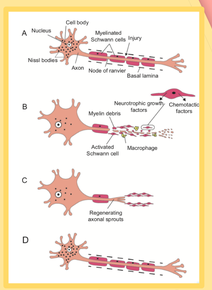

Figure 1. Schematic of neuronal axon damage and reparation with neurotrophic growth factors *(Heba et al., 2015)*

Objectives

- To determine if mesenchymal stem cells (MSCs) can be differentiated into Schwann cells with only electrical stimulus.
- To determine if MSCs exhibit biologically active paracrine activity by co-culturing them during differentiation with adult hippocampal progenitor cells

Methods

Device Production

- Circuits were inkjet-printed onto polyimide films using a Fujifilm Dimatix Materials Printer
- The ink is composed of graphene oxide, cyclohexanone, terpineol, and ethyl cellulose
- Graphene was laser annealed to increase conductivity and resilience

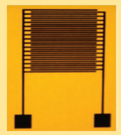

Figure 2. Graphene IDE for differentiating MSCs and AHPCs

Cell Culture

- MSCs were cultured in α-MEM supplemented with 20% fetal bovine serum, 4 mM L-glutamine, and antibiotic-antimycotic
- AHPCs were cultured in DMEM with penicillin streptomycin, glucose, N2, and bFGF
- When confluent, 200,000 MSCs and 70,000 AHPCs (GFP expressing) were seeded to their respective devices for differentiation.

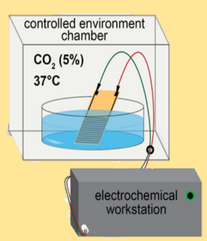

Figure 2. Schema of how electrostimulation was applied

Differentiation

- Stimulation was applied for 10 consecutive days
- Stimulus (100 mV at 50 Hz) was applied for 10 minutes each day
- During differentiation, cells were kept in their respective media; co-culture used a 50:50 ratio of media

Analyzation

Immunocytochemistry (ICC) analysis was performed to discern the degree of differentiation of MSCs into Schwann cell-like phenotypes and the degree of neurite extension of AHPCs

Results

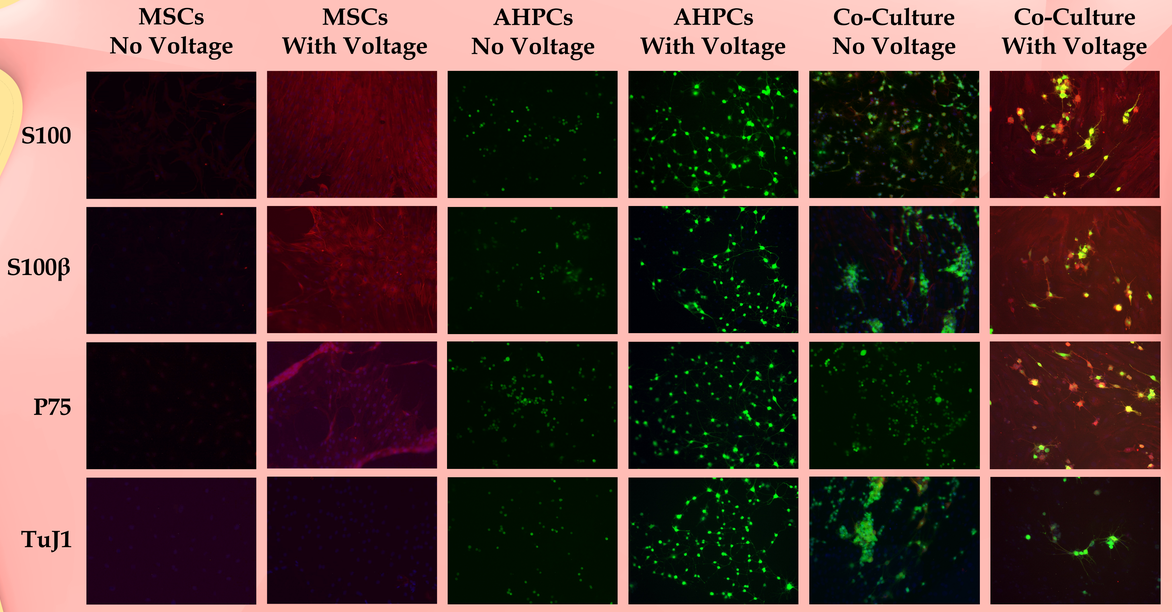

Figure 3. Immunocytochemistry (ICC) analysis suggests that electrical stimulus effectively differentiates MSCs into Schwann cell-like phenotypes independently. Applied stimulus to AHPCs causes them to extend their neurites. Furthermore, ICC shows that MSCs and AHPCs can be co-cultured and differentiated into their respective phenotypes using a single media and the same electrical stimulus.

Table 1. Quantitative analysis of ICC. Percentages represent the percentage of cells that successfully differentiated into their respective phenotypes. Quantitative results further suggest that electrical stimulus alone is effective in differentiated MSCs into Schwann cell-like phenotypes and significant neurite extension in AHPCs.

Marker	MSCs No Voltage	MSCs With Voltage	AHPCs (Neurite Extension) No Voltage	AHPCs (Neurite Extension) With Voltage	Co-Culture No Voltage	Co-Culture With Voltage
S100	~ 10%	~ 90%	~ 0%	~ 90%	~ 15% AHPC ~ 5% MSC	~ 75% AHPC ~ 90% MSC
S100β	~ 5%	~ 90%	~ 0%	~ 90%	~ 5% AHPC ~ 0% MSC	~ 70% AHPC ~ 90% MSC
P75	~ 0%	~ 90%	~ 0%	~ 90%	~ 0% AHPC, ~ 0% MSC	~ 80% AHPC ~ 90% MSC
TuJ1	~ 0%	~ 0%	~ 0%	~ 90%	~ 0% AHPC, ~ 0% MSC	~ 70% AHPC ~ 0% MSC

Conclusions

- ICC shows that electrical stimulus causes a high degree of differentiation of MSCs to Schwann cells and AHPCs to extend neurites
- ICC also shows that MSCs and AHPCs can be co-cultured and simultaneously differentiated with the same electrical conditions
- Differentiation using only electrical stimulation is significant because it avoids complications from chemical environment shifts
- Co-culture is significant because it is a biological indicator that Schwann cells derived from electrical stimulation may increase the regeneration of peripheral nerves

Next Steps

1. Paracrine activity quantification through ELISA protein assay
2. RNA analysis through PCR and western blot
3. Geometric optimization of a nerve regeneration conduit
4. Implantation in rats or mice
5. Clinical trials in humans carried out by neurosurgeons

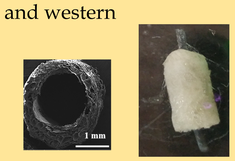

Figure 4. Examples of geometrically optimized growth conduits.

References

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Acknowledgements

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