The Influence of Therapeutic Ultrasound Stimulation on Schwann Cell Plasticity for Peripheral Nerve Regeneration

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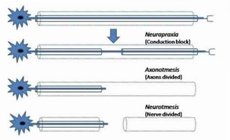
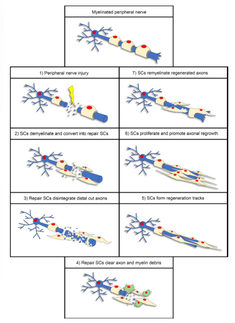
Background

- Over 67,000 Americans suffer from peripheral nerve system damage each year[1].
- Damaged peripheral nerves possess a limited amount of regenerative potential due to the presence of Schwann cells within the peripheral nerve system, which can transdifferentiate into a regenerative cell type to support nerve recovery[3].
- Low intensity ultrasound stimulation (US) has been used to promote nerve regeneration, but the exact impact of US on Schwann cells is not well defined[4].
- The effects of applying US in tandem with other microenvironmental cues such as electric stimulation has not been thoroughly investigated.



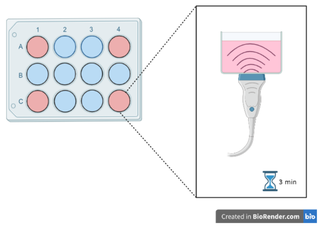
Severity of Peripheral Nerve Injuries[2]



Mechanism of Schwann Cell Mediated Nerve Repair[3]

Methods

- *Cell Culture*
  - Cells were seeded at 100 cells/mm^2 on either glass coverslips or PVDF-TrFE (polyvinylidene fluoride-trifluoroethylene) scaffolds in the 4 corners of a 12 well plate.
- *US Stimulation*
  - Experimental groups: control (no US), 0.08 W/cm^2 US, 0.8 W/cm^2 US.
  - US groups were stimulated for 3 minutes either 16 hours after seeding or 24 hours after seeding.
  - US was applied using and handheld device placed against the bottom of the culture plate
- *Proliferation Assay*
  - Cell metabolic activity was analyzed using an MTT colorimetric assay
- Im*munofluorescent Staining & Elongation Assay*
  - Cells were stained with anti-rabbit and rhodamine phalloidin to obtain F-actin labeled images.
  - Elongation was quantified using NIS elements software



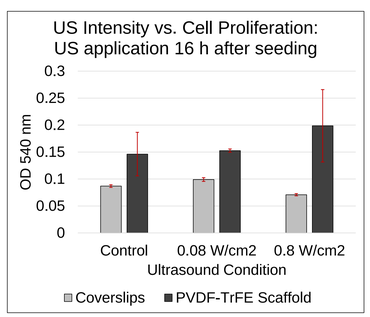
US Application Setup

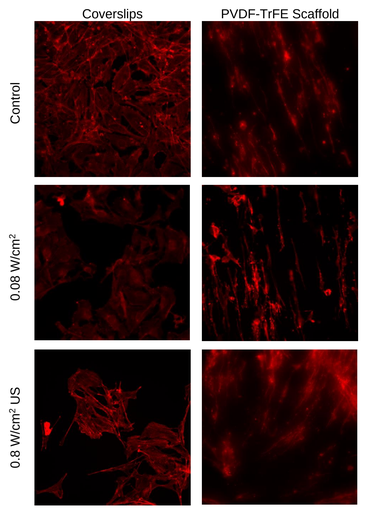
Research Objectives

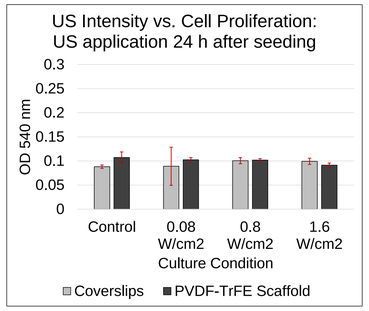
- Investigate the effect of US stimulation on Schwann cell regenerative behavior, specifically examining cell proliferation and elongation.
- Investigate the interplay between US stimulation and the piezoelectric response of a PVDF-TrFE scaffold for promoting a regenerative Schwann cell phenotype.
- Hypothesized that US stimulation would promote both cell elongation and proliferation, and that this effect of US on cells would be enhanced by culturing cells on a PVDF-TrFE scaffold.

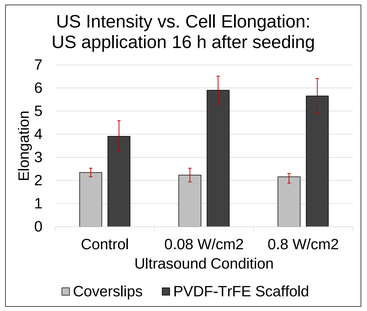
Results

- US did not affect cell proliferation.
- US did not affect cell elongation of cells cultured on coverslips.
- US significantly enhanced elongation of cells cultured on PVDF-TrFE scaffolds.
- There was no statistically significant difference in cell elongation between 0.08 W/cm2 US and 0.08 W/cm2 US samples on PVDF-TrfE scaffolds









Discussion

- Previous literature has shown low intensity US (≤1 W/cm2) may be used to induce regenerative effects in biological tissues, while our results indicate that US did not influence cell proliferation or elongation unless cells were also cultured on PVDF-TrFE scaffolds. One reason for this difference may be that we only applied US once to our cells, while other studies have stimulated cells multiple times over a longer period.
- Based on the previous knowledge PVDF-TrFE releases an electric charge after undergoing mechanical deformations, and electrical stimulation has been used to modulate Schwann cell behavior[5], our results seem to indicate that the vibrations caused by the US waves may have caused mechanical deformations in the scaffold. These mechanical deformations may have elicited a piezoelectric response, thus stimulating the cells and promoting elongation.
- A key limitation to this study was that US was applied using a handheld device rather than an immersion bath. The bouncing of US waves on the walls of plate may have caused interference between samples and decreased the accuracy of the results.

Conclusions & Future Perspectives

- US was successfully used to promote Schwann cell elongation when used in combination with a piezoelectric substrate.
- Future work should include further quantification of the piezoelectric response generative by US stimulation, and further investigation of the relationship between piezoelectric response and Schwann cell phenotype.

Acknowledgments

- All reagents, cells, and other supplies were provided by the Harris Lab at University of Cincinnati. We gratefully acknowledge the help of our advisor Dr. Greg Harris, as well as Dr. Leyla Esfandiari, the graduate students from the Harris Lab, and the graduate students from the Esfandiari Lab who contributed to this project.

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