Live and Dead Analysis for Electrical Stimulation of Adipose-derived Stem Cells

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Introduction

- Adipose-derived stem cells (ASCs) are being explored for use in neural regeneration applications because they are known to secrete factors neural-specific and wound healing growth factors and can be readily sourced by liposuction[1].
- Exogenous electrical stimulation was applied to ASCs to test if it is possible to augment pro-regenerative secretome production.
- The aim of this study is to investigate the effect of field strength and time of stimulation on ASCs viability and metabolism.

Methodology

- ASCs were obtained from a commercial source (Lonza) and grown in DMEM/F12 + 10% FBS then transferred to StemPro for electrical stimulation.

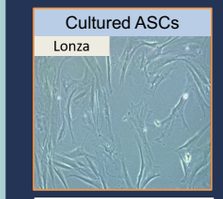


Figure 1: ASCs from commercially available source (Lonza)

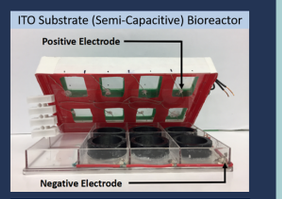


Figure 2: Indium Tin Oxide (ITO) Bioreactor

- ASCs were exposed to intermittent or continuous electrical stimulation.
- Live/Dead staining was used to determine the viability of ASCs and alamarBlue was used to determine changes in metabolism.

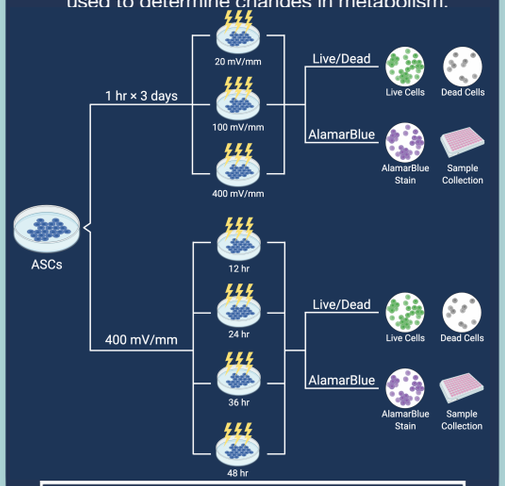


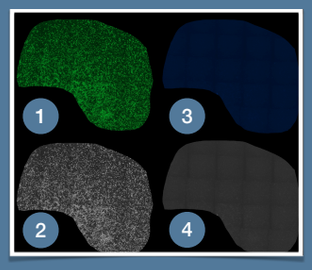
Figure 3: Schematic of methodology for assessment of viability and metabolism

Results

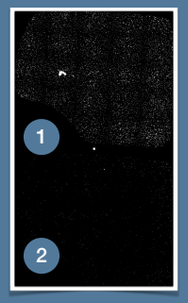
Protocol

- A novel Live/Dead analysis protocol is developed using ImageJ and MATLAB to facilitate the process

File Organization	ImageJ Macro	Region of Interest	Threshold	MATLAB	Counting
Streamline the process Identify file path in later steps	Batch Process Increase efficiency Decrease error	Exclude out of focus areas Exclude borders	Convert to binary image Exclude small artifacts	Overlap live cells and total cells to identify dead cells	Using Analyzing Particles to count the amount of total and dead cells



1. Calcein stain ROI image (live cells).
2. Split channel image for Calcein ROI for MATLAB.
3. Hoechst stain ROI image (all cell nuclei).
4. Split channel image for Hoechst ROI for MATLAB.



1. MATLAB output of Hoechst stain. Shows live and dead cells.
2. MATLAB output of *Hoechst - Calcein* cells. Shows dead cells' nuclei

Field Strength

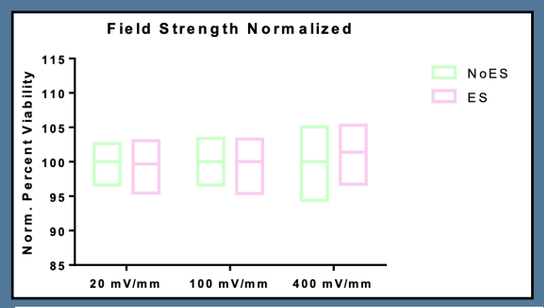


Figure 4: Live/Dead analysis on Lonza ASCs at 20, 100, 400 mV/mm field strength for 1 hr × 3 days. Intermittent stimulation at any field strength tested did not result in significant cell viability differences.

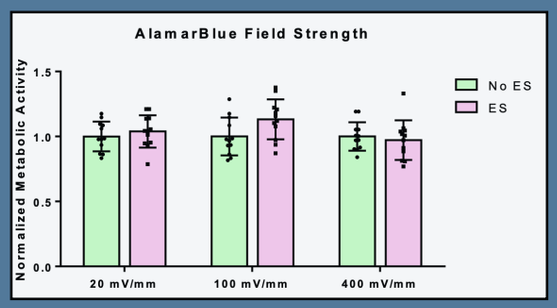


Figure 5: AlamarBlue samples on Lonza ASCs at 20, 100, 400 mV/mm field strength for 1 hr × 3 days. No significant differences in metabolic activity were detected.

Time of Stimulation

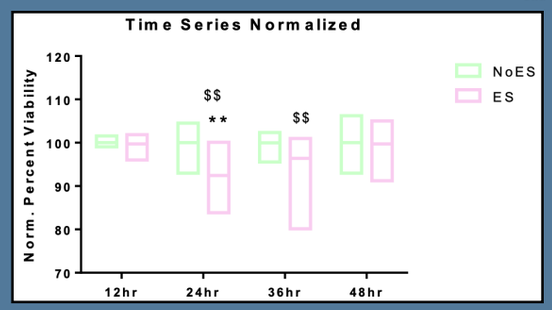


Figure 6: Live/Dead analysis on Lonza ASCs that underwent continuous electrical stimulation for 12, 24, 36, 48 hr at 400 mV/mm. $$p-value<0.05 compared to respective NoES, **p-value<0.05 compared to all other groups.

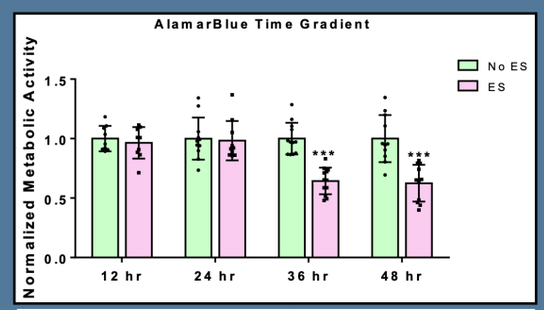
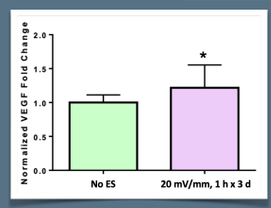


Figure 7: AlamarBlue samples on Lonza ASCs that underwent continuous electrical stimulation for 12, 24, 36, 48 hr at 400 mV/mm. There is a threshold response at 36h where there is a significant decrease in metabolic activity beyond this point. *** p-value<0.05 compared to all groups

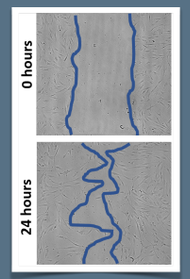
Optimizing Electrical Stimulation



- There is a significant but low up-regulation of VEGF expression at 20 mV/mm. This study aims to investigate a higher field strength and longer duration to see if there is a higher up-regulation.

Figure 8: VEGF – 20mv/mm 1hx3d, ITO bioreactor

- Most studies investigate higher field strengths and longer durations[2,3].
- The preliminary data shows that 400 mV/mm for 24hr stimulation is the highest field strength and time of stimulation before the metabolism is disturbed.
- Exploring different stimulation parameters to reach just below the metabolic disturbances for ASCs.



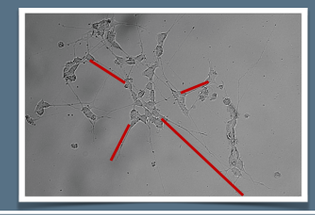


Figure 9: Dermal fibroblasts- scratch assay (left) and SY5Y- neurite outgrowth (above). These assays are used to screen secretome from electrically stimulated ASCs.

Future Work

- Troubleshoot and adjust the Live/Dead protocol as needed
- Analyze the statistics of the data

Conclusions

At 400 mV/mm for 24hr stimulation is the highest field strength and time of stimulation before the metabolism is disturbed.

There are no significant cell viability and metabolic activity differences at 400 mV/mm field strength. It is the maximum field strength that produces stable ASC survival and can be used for subsequent analyses.

The metabolic activities of ASCs are disturbed after being electrically stimulated for 36 and 48 hr. 24 hr should be used to maximize electrical stimulation for subsequent studies.

Cell viability is decreased at 24 hr and 36 hr time of stimulations. Yet, cell metabolism is not impacted at 24 hr. Further study is needed to determine if 24 hr is the optimal time of stimulation.

Acknowledgements

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References

1. **Cofano, F., et al. Int J Mol Sci, 2019, 20, 2698.**
2. **Zhang, J., et al. J Tissue Eng Regen Med 12, 2018.**
3. **Beugels, J., et al. Sci Rep 9(12076), 2019.**