TAP Milestone I Report

Abstract

EyeCell's goal is to revolutionize the treatment of retinal degeneration and vision loss by using a three component platform of (1) bioelectric microstimulator, (2) refillable, programmable repeat delivery infusion pump and (3) stem cell, growth factor and matrix based composition for complete eye regeneration. In order to establish proof of concept for the bioelectric microstimulator, human eye tissue was stimulated and the response in protein expression was determined by qPCR. It was shown that upregulation of gene expression for four of the five desired genes occurred as a result of gene-specific bioelectric stimulation on the cadaveric eye tissue. Results for the fifth gene were inconclusive. Using this information, EyeCell will now prepare for therapeutic use in animal trials as it has conducted a successful proof-of-concept in vitro validation of the bioelectric stimulation signals.

Introduction

Over 200 million people worldwide suffer from blindness or vision impairment. Visual loss and blindness can be caused by diseases related to retinal degeneration and dysfunction such as age-related macular degeneration (AMD), retinitis pigmentosa (RP) and Stargardt's disease, among others. AMD affects nearly 170 million people. The reported global cost due to retinal degenerative disease in $343 billion. These numbers are expected to grow with an aging global population.

EyeCell seeks to provide a safe and effective product to allow for complete eye regeneration. The platform technology for EyeCell is comprised of three components- (1) a micro stimulator providing bioelectric controlled regeneration promoting protein expressions, (2) a re-fillable, programmable repeat delivery capable infusion pump and (3) a stem cell, growth factor and matrix based mixed composition optimized for ocular tissue regeneration.

Our patented micro stimulator device delivers precise bioelectrical signals to tissue cells directing them to release specific proteins at the exact time they are needed. By applying different signals we can stimulate the controlled release of proteins that are specific to stem cell recruitment and retinal regeneration. In the past, it has been shown that our microstimulator is capable of promoting IGF-1, a gene which plays a central role in rescuing retinal cells after release by bioelectric signaling[1]. EyeCell via Leonhardt Ventures has patent for bioelectric signals for the release of thirteen different regeneration promoting protein expressions including stem cell homing signal, stem cell controlled proliferation and controlled differentiation.

In order to establish proof of concept and prepare for animal trials, we tested the response of samples taken from human cadaver eyes to five different bioelectric signals

designed to promote the expression of regeneration related proteins. The results of this experiment will inform the procedure used in future animal trials to determine the safety and efficacy in using bioelectric stimulation to promote retinal regeneration as a curative solution to visual impairment and blindness.

Materials and Methods

Two fresh, healthy human cadaver eyes, provided through the Moran Eye Center in conjunction with the Utah Lion's Eye Bank were enucleated and stored at -80°C to preserve tissue structure. They were then dissected by removing the optic nerve and the anterior portion on the eye after which the lens and vitreous humor were also removed. Both eyes were cut into three pieces (six total). The resulting samples were comprised of the sclera, choroid and retina layers.

Five of the samples were then stimulated for 20 minutes each with different electrical signals designed to promote the release of one specific target protein (Table 1).

Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
VEGF	SDF-1 (CXCL12)	IGF1	CRYAA	ELN	Control

Table 1: Description of type of gene specific bioelectric stimulation

Stimulation was performed by placing the tissue sample between two electrodes that were connected to a GRASS S88 Square Pulse Stimulator as shown in Figure 1. The sixth sample was immediately frozen and served as a control.

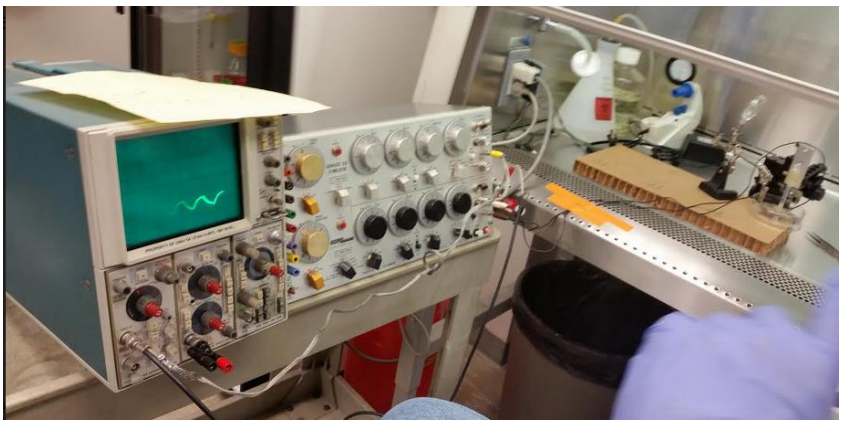

Figure 1: Setup of the equipment and sample used for bioelectric stimulation.

After stimulation, all six samples were immediately taken to the University of Utah DNA Extraction CORE facility where mRNA was isolated from the samples (PureLink RNA Mini Kit,

Thermofisher), tested for quality and reverse transcribed to generate cDNA (RT^2 First Strand Kit, Qiagen). Real time PCR was performed for each trial using RT^2 SYBR Green ROX qPCR Mastermix (Qiagen) combined with the 96-well RT^2 Human Macular Degeneration Profiler PCR Array plate (Qiagen). These plates tested for 84 different genes that are associated with retinal regeneration as well as quality control and housekeeping genes. Results from the real-time PCR trials were analysed using software provided by Qiagen through the GeneGlobe Data Analysis Center and compared to the control group to determine to what degree target genes, and others, were upregulated by bioelectric stimulation.

Results and Discussion

Results of the study can be summarized in Table 2 for our specified genes of interest. However, a more complete described and analysis of the results are shown in Appendix 1.

Signal:	**VEGFA**	**SDF1 (CXCL12)**	**CRYAA,CRYAB**	**IGF1**	**ELN**
Fold-Change of expressed cDNA (compared to control):	1.10	64.91	28.92, 5.59	N/A	N/A

Table 2: Summary of the real time PCR results for genes of interest.

As shown in Table 2, three of our genes of interest were upregulated, most notably SDF1 and CRYAA, which had significant upregulation and contained 64.91 and 28.92 times the amount of cDNA found in the control sample. Unfortunately, the results for IGF1 and ELN were not conclusively determined, as there wasn't sufficient cDNA harvested from either the sample or control tissue to provide meaningful data.

In addition to modifying expression levels for our specified genes, the electrical signals also had effects on other genes in the plate array. The most potent signal, it appears, came from the signal used to stimulate SDF1 (CXCL12), as 60 other genes were also upregulated (the highest, IL6, was upregulated over 2,000 fold!). This example, as well as the overall data shown in Appendix validates our position that mRNA and consequent gene expression levels can be significantly altered and upregulated by bioelectric signals.

Conclusion

The data presented in Table 2 and in Appendix 1 supports our theory of controlled gene expression for bioelectric stimulation directed at specific genes. Although two of the five genes of interest did not show meaningful data, we do not have reason to believe it was due to the effects of bioelectric stimulation since even the levels of cDNA on the control plates were not enough to collect meaningful data for these genes. We hypothesize that the lack of cDNA for these genes stemmed from a lack of mRNA collected from the eye tissue, which could have

been caused for a variety of reasons due to the high rate of degradation of mRNA. However, it could very well be possible that the eye tissues simply weren't producing a high enough amount of IGF1 or tropoelastin (ELN) to begin with, so although upregulation may have occurred due to bioelectric stimulation we would have arrived at inconclusive results due to the very low number of mRNA copies produced.

Overall, we consider the results of this experiment a success for validating our proof-of-concept phase of our overall experiment and feel they accomplish the objectives of Milestone I of the USTAR TAP grant. These findings are crucial to our moving into the next phase in the development of this technology which is small animal studies (likely rats), comprising Milestones II & III.

References

1. Transcorneal electrical stimulation rescues axotomized retinal ganglion cells by activating endogenous retinal IGF-1 system. Morimoto T, Miyoshi T, Matusda S, Tano Y, Fujikado T, Fukuda Y. *Invest. Ophthalmol Vis Sci.* June 2005. Vol 46, No. 6, pg 2147-55.