Evaluation of Corneal Endothelium Barrier Function on Electrospun Scaffolds

Garyn Levitan[1], Meiji Wang[1], Nasif Mahmood[1], Michael Daniele[2], Jessica M. Gluck[1]
[1]Textile Engineering, Chemistry and Science
[2]Electrical and Computer Engineering
North Carolina State University

Statement of Purpose:

Vision is essential to navigating the world around us. Annually, 2.8 million people require a corneal transplant; however, only 40,000 actually receive one [1]. While currently the best available procedure, corneal transplants from donors have many disadvantages for patients other than lack of accessibility. These transplants could potentially be one of low quality due to the age of the donor. Treatments utilizing tissue engineering are being researched to identify an alternative procedure. In order to produce these alternative treatments in an effective way, they must be tested accordingly. Our goal is to produce corneal endothelium scaffolds that have a barrier function similar to a healthy corneal endothelium. In order to understand this barrier function, we must measure the electrical resistance of these scaffolds. Currently the “gold standard” for functional testing is the Trans-Endothelial Electrical Resistance (TEER) test. The commercially available TEER test generates an alternating current of electrical signals. Our current findings using this test indicate that it exhibits variability in measurement results. TEER also lacks reliability and repeatability as a testing method. This test must be optimized in order to reduce the variability in results. Our research has now shifted focus onto performing the TEER test with the inclusion of scaffolds.

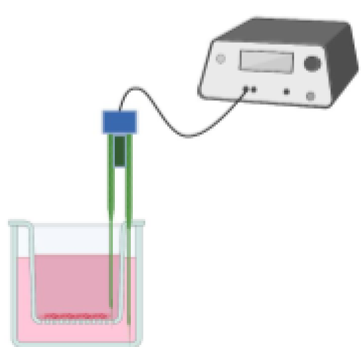
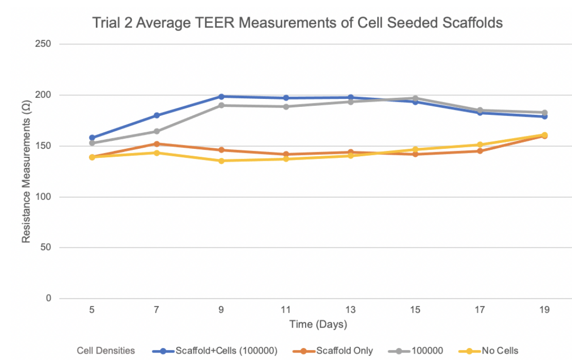


Figure 1. TEER Test Setup Using Chopstick Electrodes

Methods:

Preliminary testing determined the appropriate cell seeding density. Corneal endothelial cells were prepared and seeded onto the inserts of Transwell plates. Different seeding densities were used to find the optimum density: 3x wells (10,000, 20,000, 50,000, 100,000, and Media only).The resistance across the cell monolayers in the well inserts were measured every other day using the Millicell ERS-2 Voltohmmeter. Resistance is measured by inserting the tips of an electrode pair into the well and insert of the Transwell plate. After determining the optimal cell seeding dentistry of 100,000cells/well, we then focused on optimizing the testing approach by incorporating an electrospun scaffold containing carbon nanotubes.

Results:



The addition of the conductive scaffolds does not adversely affect the testing method itself. The resistance measurements of the scaffolds+cells are very similar to the cell only measurements. This shows that the highly conductive scaffolds did not make a major difference in results. The scaffold-only measurements are almost identical to the well with no scaffold or cells. The CNT scaffolds showed high result fluctuation during each measurement.

Conclusion:

The TEER test has the potential to show promising data if the following adjustments are made to the procedure: the addition of scaffolds fabricated with a less conductive, conductive polymer Polyaniline (PANI) to provide consistent results without interference from the scaffold, and also to attempt TEER with a stationary electrode pair to eliminate result fluctuation. The use of PANI will allow the scaffold to closely mimic the native environment of the endothelium since there is a presence of ions in this region of the cornea.

References:

[1] Ghezzi CE, Rnjak-Kovacina J, Kaplan DL. Corneal Tissue Engineering: Recent Advances and Future Perspectives. *Tissue Engineering Part B: Reviews*. 2015;21(3):278-287. doi:10.1089/ten.teb.2014.0397.