DEVELOPMENT OF NOVEL BROAD-SPECTRUM ANTIMICROBIAL CORNEAL TISSUE STORAGE SOLUTIONS

Endophthalmitis is a severe condition caused by inflammation of the ocular cavity and is a significant complication of corneal transplant procedures, often being caused by microbial contamination of the donor tissue. While corneal storage solutions used in Europe contain both antibacterial and antifungal agent(s), similar products do not exist in the US market. Currently, in the US, the most commonly used corneal tissue storage solution is Optisol-GS (Bausch & Lomb, Inc.), which contains two broad-spectrum antibiotics: gentamicin and streptomycin. With the proportion of fungal infections post-keratoplasty on the rise, there is a clear need for the development of new corneal tissue storage products. For these studies, iFyber evaluated the use of 13 FDA-approved antifungal agents, both alone and in combination, to identify the most promising candidate formulations for the development of an Optisol-GS-based corneal storage solution. High-throughput 96-well plate-based screening was used to assess the antifungal efficacy of the combination treatments. This checkerboard assay design allows for simultaneous assessment of numerous concentration combinations. A 3D human corneal tissue model (EpiCorneal™, MatTek Corporation) was then used to screen the lead corneal storage solution formulations for potential effects on corneal tissues during storage.

Figure 1. The MTT assay measures mitochondrial activity of basal and suprabasal cells where viable cells reduce the MTT dye (yellow) to formazan (purple). A higher readout represents better cell viability. Data are normalized to the negative control and the yellow line marks the desired 100% viability. While the positive control (0.3% Triton X-100 for 3 h) caused a decrease in tissue viability, none of the formulations evaluated showed negative effects within this assay, even after an 8-day exposure time.

Figure 2. The lactate dehydrogenase (LDH) assay measures cytotoxicity through enzymatic activity of LDH that is released from damaged cells – conversion of iodonitrotetrazolium (INT) into formazan. Note that the desired output here is no detectable activity. None of the formulations evaluated showed appreciable levels of LDH activity at 37°C, while at 4°C Formulation 3 showed some LDH activity. Interestingly, the positive control output (0.3% Triton X-100) was significantly lower at 4°C than 37°C samples.

Figure 3. Tissue barrier properties were evaluated with the Lucifer Yellow (LY) leakage assay. This assay uses a fluorescent dye, normally not taken up by viable cells, and not transported transcellularly. This assay was used to confirm that epithelial cells express tight junction proteins, forming a diffusion barrier. Lack of the barrier results in an increase in the fluorescence readout in the medium below the tissue. Note that in this assay, the goal is to have minimal readout which would indicate that the tissue still retains its barrier function. Based on these results, Formulation 1 showed decreased barrier function as compared with 2 and 3. Interestingly, the data were the same for both infected and non-infected tissue.