Development and Optimization of an In Vitro BBB Model for High-Throughput Screening of Modulators of the Barrier Function

H. Qosa, L. Mohamed, Y. Batarseh, K. El Sayed, J. N. Keller, A. Kaddoumi
University of Louisiana

Purpose
Blood brain barrier (BBB) is a dynamic interface that maintains brain homeostasis and protects brain from free entry of chemicals, toxins and drugs. Barrier function of BBB is maintained by capillary endothelial cells that physically separate brain from blood. Different cell-based models were developed to study BBB biology. However, available models are complex and their use for high-throughput screening (HTS) has proven to be challenging. Therefore, the aim of this study was to develop a reliable and sensitive HTS-BBB model to screen for large number of chemicals for their effect on the barrier functions of BBB.

Methods
The mouse brain endothelial cells bEnd3 were used in these studies. Cells were grown on HTS 96-well transwell inserts in culture media for several days. Lucifer yellow permeation assay was utilized to assess the integrity of bEnd3 monolayer during model optimization and compound screening. Next, optimized model was used for screening of 1500 compounds for their effect on the barrier function of bEnd3 monolayer.

Results
A series of experiments aiming to attain a maximal barrier function for bEnd3 monolayer were performed. Cells grown on polycarbonate membrane with pore size of 3 µm, coated with 30 µg/ml fibronectin as a basement membrane substituent was determined as optimal to enhance bEnd3 cells growth and to form a tight monolayer valid for permeation assay. Z' factor as a measure for HTS performance demonstrated a robust and valid model. This model was then used to screen 1500 compounds that include FDA approved drugs and investigational compounds for their effect on the barrier function using Lucifer Yellow as a permeability marker. This primary screen identified 66 compounds as disruptors and 54 compounds as enhancers of the barrier function of bEnd3 monolayer.

Conclusion
This study offers for the first time an endothelial cell-based BBB model for HTS that effectively represents the barrier function of BBB endothelium. This platform was successfully used for high-throughput screening of compounds for their modulatory effects on the barrier function of BBB endothelium.