# Selection of optimal transwell plates to evaluate intestinal and blood-brain barrier permeation of novel compounds

Naveena Murugan
Mentored by Dr. Heidi Hoard-Fruchey

## Introduction

The life expectancy of the world population is steadily increasing, but living longer comes with health problems requiring medical treatment. The transwell culture system is used to create *in vitro* biological barriers for prediction of therapeutic drug permeation. Transwell inserts are produced with different materials and membrane pore sizes. The purpose of this study was to evaluate commercially available transwell insert types to optimize permeability assays of intestinal and blood-brain barrier models. In this project, eight different transwell inserts were evaluated through series of tests analyzing their compatibility for creating *in vitro* models of biological barriers. They were tested for their transendothelial electrical resistance (TEER), Lucifer yellow (LY) and standard compound apparent permeation ($P_{app}$) properties, and cell line compatibility with the Madin Darby Canine Kidney (MDCK) and multi-drug resistance gene 1 (MDR1) transfected MDCK cells which model the selectively permeable intestinal and blood-brain barriers, respectively (Irvine et al., 1999). Compatible transwell inserts and cell lines have the potential to provide more effective information for the development of therapeutic drugs.

## Materials and Methods

Part I: TEER and LY evaluation on blank membranes.

The compatibility of the membrane material and pore sizes with *in vitro* modeling of the intestinal and blood-brain barriers was evaluated by TEER using the Manual Millicell ERS2® system and subsequently LY assays measuring fluorescence in the wells using the SpectraMax 3® fluorometer. The following inserts were evaluated: Millicell (M.) Control, HTS, M. PET, Corning Costar Polycarbonate (CC PolyC), CC Collagen, M. Polystyrene (PolyS), CC Polyester (PolyE), and Falcon Corning (FalconC). Small TEER values and large percent transmission of LY indicate membranes with high permeability. Three poorly performing inserts were eliminated from the study based on the TEER (Graph 1) and LY (Graph 2) assays.

Part II: Atenolol (low permeability), Propranolol (high permeability), and Prazosin (Moderate permeability) evaluation on blank membranes.

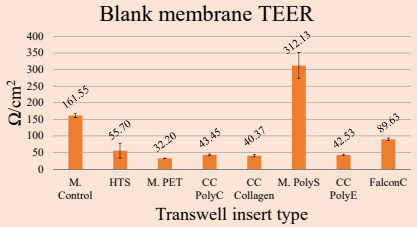
With the remaining five transwell inserts (M. Control, HTS, M. PET, CC PolyC, and CC Collagen), standard compounds, atenolol, propranolol, and prazosin were run in triplicate and relative concentration was measured by mass spectrometry for permeability evaluation. $P_{app}$ values were calculated and compared to the theoretical maximum permeability values. Two inserts were eliminated based on poor permeability values (Graph 3).

## Materials and Methods (cont.)

Part III: Cell density optimization of MDCK and MDR1-MDCK cells and standard drug permeation assays.

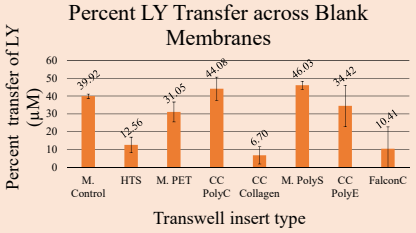
With the remaining three inserts (M. Control, HTS, and M. PET), the compatibility of the MDCK and MDR1-MDCK cells were evaluated by analyzing the growth of the two cell lines at a range of seeding densities (50–300 K cells/cm² and 100–400 K cells/cm², respectively). The cell barriers grown on the inserts were evaluated on the third day using TEER (Graph 4 and 5). Optimal seeding densities were chosen for each insert-cell type, and the standard compounds were assayed for $P_{app}$ values and efflux ratios (Table 1).

## Results

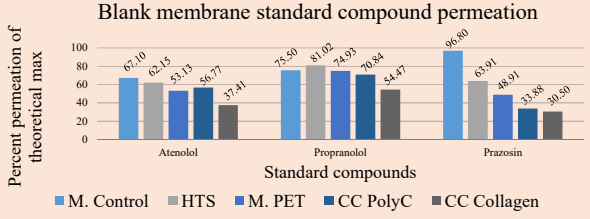



Graph 1 (left): The standard deviations of the TEER ($n = 3$) reflect the consistency of the transwell inserts. Test inserts with $p$-values < 0.05 when compared with M. Control with a Bonferroni's Multiple Comparison ANOVA Test and TEER greater than M. Control were eliminated from the study.

Graph 2 (right): The standard deviations of the LY ($n = 3$) reflect the consistency of the inserts. Test inserts with $p$-values < 0.05 when compared with M. Control with a Bonferroni's Multiple Comparison ANOVA Test and LY less than M. Control were eliminated from the study.




Graph 3 (below): The inserts with markedly lower percent of theoretical maximum transfer ($n = 3$) of the standard compounds over blank membranes compared to M. Control were eliminated from the study.




## Results (cont.)

Graph 4 (left) and 5 (right): The optimal seeding densities of the MDCK and MDR1-MDCK cells were identified as the highest TEER values ($n = 3$) corresponding to optimal conditions. The standard deviations show variability in barrier formation.

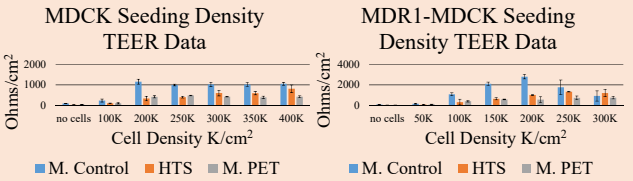



Table 1 (below): The standard deviation shows the variability of drug permeation in *in vitro* models. The data for MDCK cells is not shown. *$P_{app}$ < 2 Low; 2-20 Moderate; > 20 High Permeability **Efflux ratio > 2.5 means compound is effluxed

| MDR1-MDCK standard compound permeation | | | |
|---|---|---|---|
| Inserts | Drugs | $P_{app}$* | Efflux Ratio** |
| M. Control | Atenolol | 0.93 ± 0.01 | 1.35 |
| | Propranolol | 45.14 ± 0.65 | 0.9 |
| | Prazosin | 7.67 ± 0.19 | 8.33 |
| HTS | Atenolol | 1.80 ± 0.04 | 0.74 |
| | Propranolol | 53.64 ± 0.17 | 0.6 |
| | Prazosin | 12.54 ± 0.34 | 8.81 |
| M. PET | Atenolol | 1.04 ± 0.06 | 5.12 |
| | Propranolol | 45.58 ± 0.1 | 1.00 |
| | Prazosin | 12.06 ± 0.19 | 8.00 |

## Conclusions

The purpose of this study was to optimize the transwell culture systems for intestinal and blood-brain barrier permeability assays so that future permeation studies can incorporate transwell inserts that are compatible with the cells and drug compounds. The results show that the HTS and M. Control inserts are both optimal for creating *in vitro* models of the intestinal and blood-brain barriers. The M. PET offered optimistic results, but further evaluation through repetition of the standard drug assays is needed for confirmation on its viability. Moreover, these results provide alternative and effective transwell systems for permeability assays for the development of novel therapeutic drugs.

## References

Irvine, J. D., Takahashi, L., Lockhart, K., Cheong, J., Tolan, J.W., Selick, H.E., & Grove, J.R. (1999). MDCK (Madin-Darby Canine Kidney) Cells: A Tool for Membrane Permeability Screening. *Journal of Pharmaceutical Sciences, 88*(1), 28-33. doi: 10.1021js9803205