Increasing Throughput of Chemotaxis, Invasion and Drug Transport Studies with Permeable Supports

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Abstract

The use of *in vitro* cell models is widely applied in the drug discovery process. A recent trend has been to introduce the use of these models earlier in the process. In fact, in some instances, these assays are being used as part of the primary or secondary library screening process. To meet the demands of this level of throughput, a robust, high throughput process must be established. We compared the performance of a 96 well based permeable support assay system to a 24 well format based process. We show that chemotaxis, invasion and drug transport assays, performed in this high throughput configuration are equivalent to those done in the lower throughput 24 well systems. This results in a higher number of samples capable of being tested per day with lower amounts of reagents necessary for each test condition. Furthermore, the variation in signal from well to well is reduced and a lower percentage of wells are discarded for lack of performance. These results indicate that meeting the demands of a high throughput screening environment can be reliably achieved in a 96 well based format.

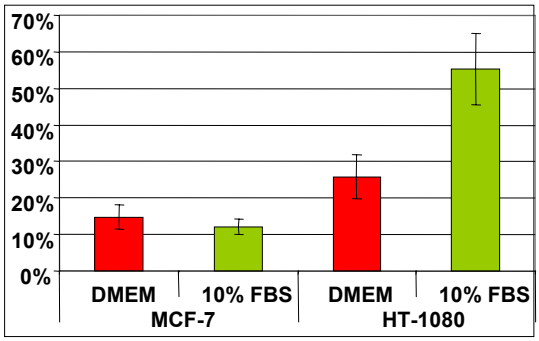
Methods

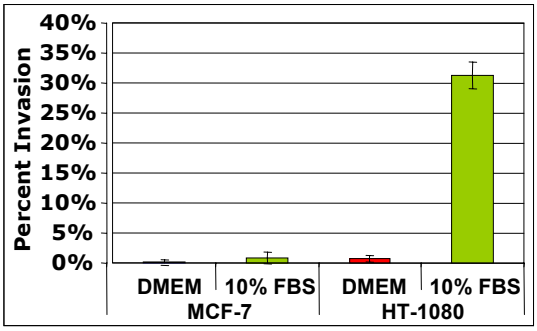
Cells: Caco-2 and MDCK cells were maintained in Iscove's Modified Eagles Medium (IMDM) supplemented to 10% fetal bovine serum (FBS), 4 mM glutamine and 1% penicillin/streptomycin. MCF-7 and HT-1080 cells were maintained in Dulbecco's Modified Eagle's Medium supplemented to 10% FBS and 4 mM glutamine.

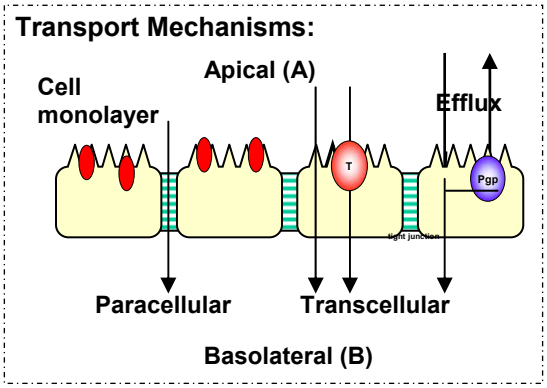
Monolayer Analysis: At the end of the culture period each plate was tested for monolayer integrity by transepithelial electrical resistance (TEER) and lucifer yellow (LY) rejection. For TEER measurements, media was changed and TEER values detected using a World Precision Instruments EVOM voltmeter. For LY transport, each insert was washed with HBSS (plus Mg^{++}/Ca^{++}) 3 times. Lucifer yellow at 60 mM was added to each apical surface of the insert and placed in a receiver plate containing 1X HBSS; 1% dimethylsulfoxide (DMSO). Plates were incubated at 37°C/5% CO_2 for 1 hr while shaking. Apparent permeability of LY was determined by comparing the final concentration in both the apical and basolateral compartments.

Transport Studies: Caco-2 cells were seeded at $3.6e^4$ cells/cm^2 and grown for 21 days at 37°C; 5% CO_2 with media changes every 2-3 days. MDCK cells were seeded at $1.07e^5$ cells cells/cm^2 and grown for 5 days at 37°C; 5% CO_2. All compounds were used at 60 mM, except for Rhodamine 123 which was used at 50 mM. Assays were performed as above for LY. For B to A transport compounds were placed in the lower chambers at the start of the assay. Samples were collected and analyzed either by reverse phase HPLC or LC/MS. Apparent permeability was calculated as above.

Chemotaxis/Invasion assays: Cells were incubated in medium without serum for 24 hr prior to seeding into HTS Transwell®-96 permeable supports. Cells were harvested and 50,000 cells were seeded into each well in serum free medium. Serum containing medium (10% FBS) was added to the receiver wells as chemo-attractant. Cultures were incubated at 37°C; 5% CO_2 for 24 hr. After incubation wells were washed and cells were stained with Hoechst 33342 and read in a fluorescent plate reader. Percent cells was calculated as the number of cells that moved to the lower side of the Transwell® insert as a fraction of the total cells seeded. Standard curves for both cell types were established for each assay.





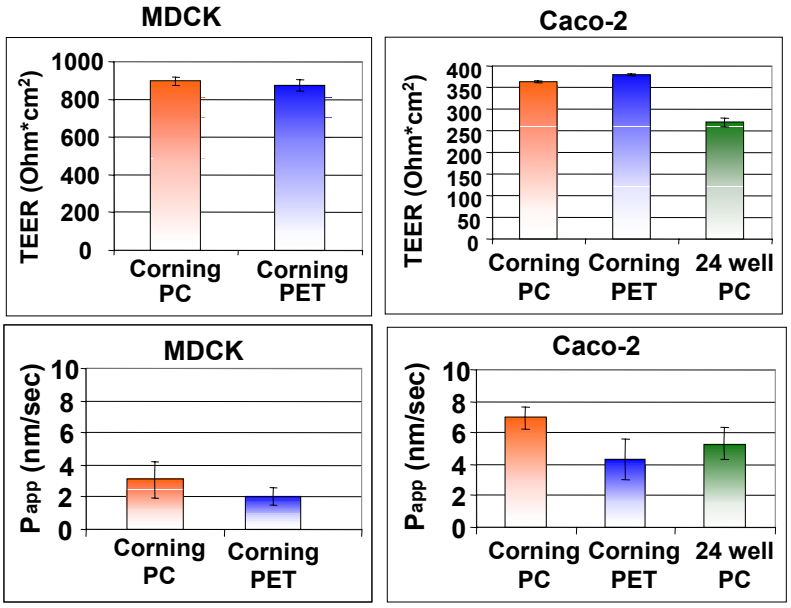


Drug Transport Models

Permeable supports are frequently used as an *in vitro* model for evaluating absorption, permeability and efflux transport properties of drug candidates. Such evaluations, as part of ADME-TOX screening, are often performed in a 24-well format. Recent advances in combinatorial chemistry and genomics have generated an unprecedented number of compounds needed for such testing, and have led to an increasing need for higher assay throughput. The adjacent figure illustrates the 4 main ways compounds can be transported across cell monolayers.

Results

Figure 1: TEER and LY analysis of MDCK and Caco-2 cultures. The HTS Transwell®-96 permeable supports utilize either a 0.4 mm polycarbonate membrane (Corning PC) or a 1.0 mm polyester membrane (Corning PET). Bars represent the average ± the standard error from at least 3 experiments. TEER and apparent permeability of LY was similar for both cell types on HTS Transwell®-96 permeable supports. In general, the PET membrane is slightly better at creating intact monolayers than the PC membrane.



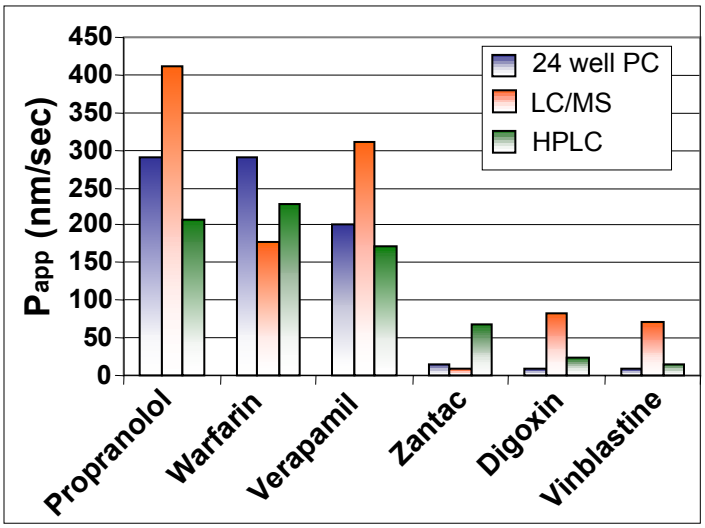
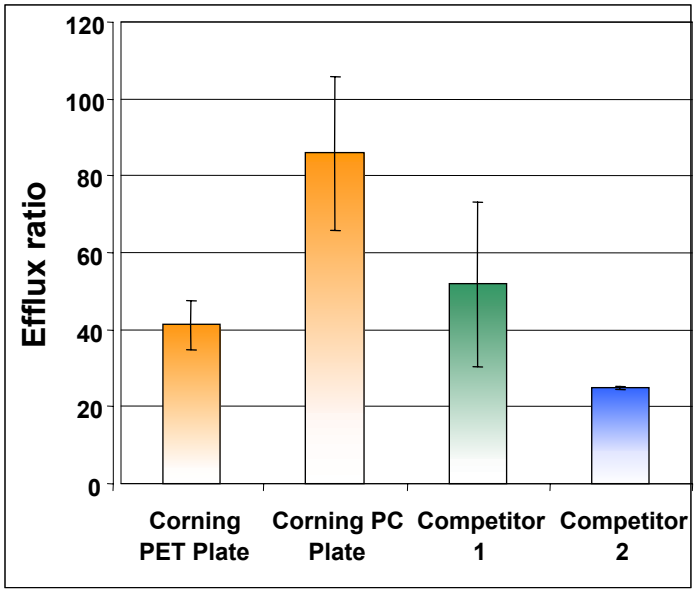


Figure 2: Drug Transport analysis of known compounds. Several compounds of known permeability were tested for transport in Caco-2 cultures. After incubation for transport, samples were collected and analyzed via either HPLC or LC/MS. In these studies, HTS Transwell®-96 permeable supports with the polycarbonate membrane were used. Apparent permeability shown is for the A>B direction. Each compound was tested in at least 6 wells in at least 3 independent experiments.

Figure 3: Evaluation of Rhodamine 123 efflux in Caco-2 cells. A to B and B to A transport was calculated as described for LY. Two 96 well based competitor plates were also evaluated for efflux. Each experiment was performed 3 separate times with 32 wells evaluated for each direction of transport for each plate. Bars represent the average ± standard deviation.



Results cont.

Figure 4: Chemotaxis in HTS Transwell®-96 permeable supports. Both non-invasive (MCF-7) and invasive (HT-1080) cells were seeded into wells of a HTS Transwell®-96 permeable support with an 8 mm pore size PET membrane. Four wells per plate were used for each condition tested. Results are the average ± the std. dev. of at least 4 experiments. A statistically significant greater amount of HT-1080 cells migrated to the lower surface of the HTS Transwell®-96 permeable supports in response to FBS stimuli as compared to MCF-7 cells ($p < 0.01$).

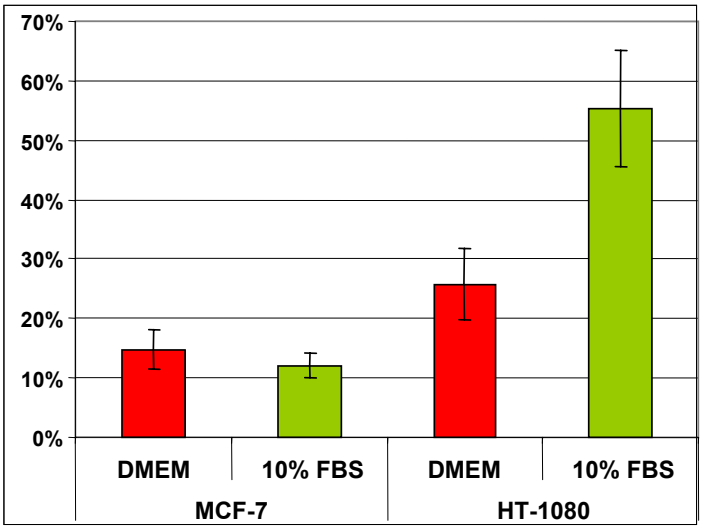
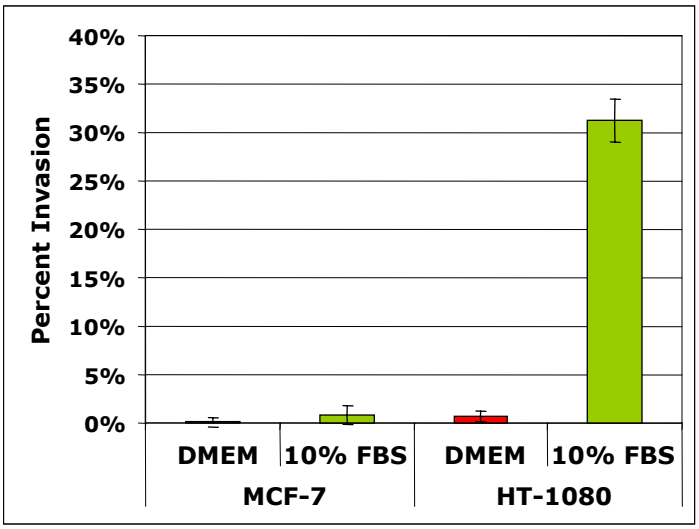


Figure 5: Invasion in HTS Transwell®-96 permeable supports. HTS Transwell®-96 permeable supports with an 8 mm pore size PET membrane were pre-coated with a basal membrane extract to occlude pores. Sixteen wells per plate were used for each condition tested. Results are the average ± the std. dev. of at least 4 experiments. A statistically significant greater amount of HT-1080 cells invaded to the lower surface of the HTS Transwell®-96 permeable supports in response to FBS stimuli as compared to MCF-7 cells ($p < 0.001$)



Conclusions:

- There is no difference in Caco-2 monolayer differentiation between 24 well and HTS-96 permeable supports
- There is no difference in Caco-2 monolayer differentiation between the PC and PET membranes
- Corning polycarbonate plates exhibit the greatest efflux ratio, possibly due to its larger surface area
- Amount of compounds required to perform transport study is 25-60% less in HTS Transwell®-96 permeable supports as compared to 24 well Transwell® permeable supports.
- Chemotaxis and Cell Invasion assays can be easily miniaturized to a 96 well format