Trying to Solve the Organ Shortage Crisis:
The Effects of Sphingosine-1-Phosphate (S1P) and Its Related Drugs on the Leakiness of a Blood Vessel Lining Model

Boonklang P.*, Wilkins G., Ali S.

BSc (Hons) Biomedical Sciences, School of Biomedical Sciences Student ID:160067853 Email: p.boonklang2@newcastle.ac.uk

Newcastle University Institute of Cellular Medicine

NHS National Institute for Health Research

Blood and Transplant Research Unit in Organ Donation and Transplantation

wellcome

Background

In 2017-2018, 5% of retrieved organs were not transplanted as they were deemed as unsuitable. This might have been due to damaged blood vessels. The aim of this project is to investigate if S1P molecule and its related drugs can improve the integrity of the blood vessel lining model or not. This can potentially contribute towards greater availability of suitable donor organs and shorter waiting lists in the future.

The project has 3 key experiments that include two pre-optimised cell culture models which are simplified representations to the real-world systems. These are:

1. **HMEC-1**: This is the **main interest** of the project as it is a blood vessel lining cell model that expresses targets for S1P and the drugs.
2. **Caco-2**: a cell model that is a very good barrier and has no target for S1P and the drugs. It is used as a positive control *i.e.* when receives treatments, it gives reliable known results to be used for comparisons with HMEC-1.

Based on literature [1,2], the effect of each drug molecule on the barrier integrity of a blood vessel lining was hypothesised as following:

	Improve	Worsen
S1P	✓	
CYM5442	✓	
CYM5541		✓
TY52156	✓	

Objective (1)

To investigate the growth patterns of the two models, HMEC-1 and Caco-2, over the course of 7 days

Method (1)

- HMEC-1 and Caco-2 cells were grown onto Transwell® inserts.
- A Transwell® insert was taken for cell fixation each day for 7 days.
- A medical staining technique was used to distinguish cells.

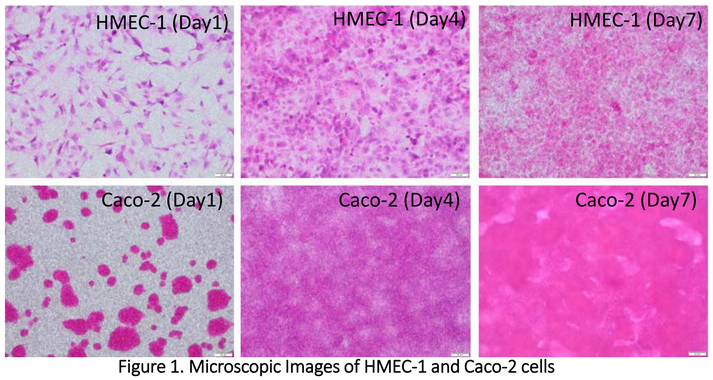


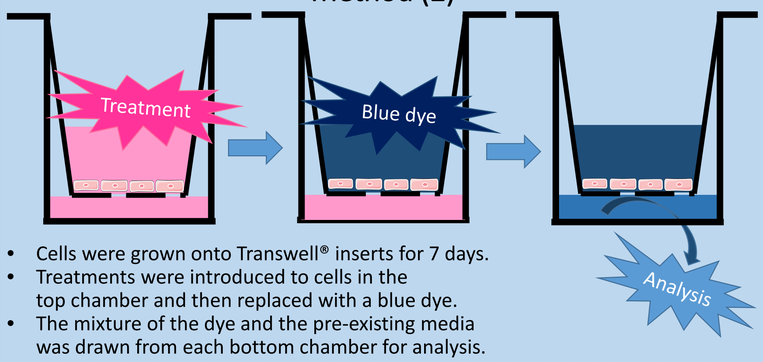
Figure 1. Microscopic Images of HMEC-1 and Caco-2 cells

- Over the course of 7 days, HMEC-1 and Caco-2 grew to cover the entire surface.
- HMEC-1 grew to form a monolayer while Caco-2 overlapped and formed multilayers.
- This suggests that HMEC-1 is a good model for blood vessel lining tissues as a lining tissue is a monolayer and HMEC-1 resembles this.

Objective (2)

To investigate if S1P and its related drugs have any effect on the permeability of the HMEC-1 model

Method (2)



- Cells were grown onto Transwell® inserts for 7 days.
- Treatments were introduced to cells in the top chamber and then replaced with a blue dye.
- The mixture of the dye and the pre-existing media was drawn from each bottom chamber for analysis.
- If the barrier integrity of the model is improved, the dye will leak less; thus, the lower the readings.

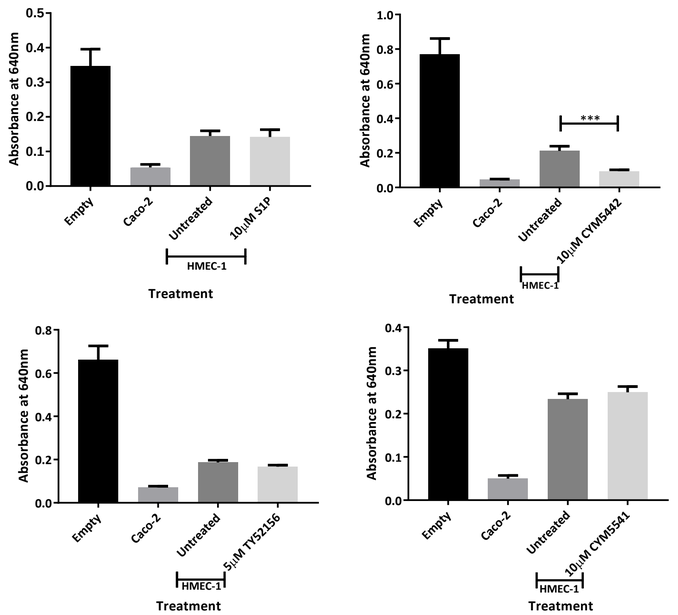


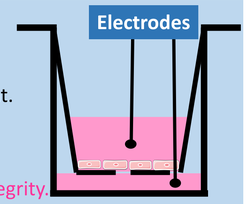
Figure 2. Absorbance values at 640 nm when treated with S1P and the drugs

- S1P had no effect on the permeability of HMEC-1 while CYM5442 significantly improved it ($p \leq 0.001$). (Number of repeats =3)
- There was a slight improvement of the barrier integrity when HMEC-1 was treated with TY52156 but slight worsening with CYM5541. However, these changes are not significant. (Number of repeats =3)

Objective (3)

To investigate electrical resistance across the cell layers of Caco-2 and HMEC-1 when treated with S1P and different drugs

Method (3)



- Electrodes were placed in both chambers of the Transwell®.
- The resistance was measured before and after each treatment.
- The readings were used to find the unit area resistance of each Transwell®.
- These were carried out in both models, Caco-2 and HMEC-1.
- The higher the electrical resistance, the better the barrier integrity.

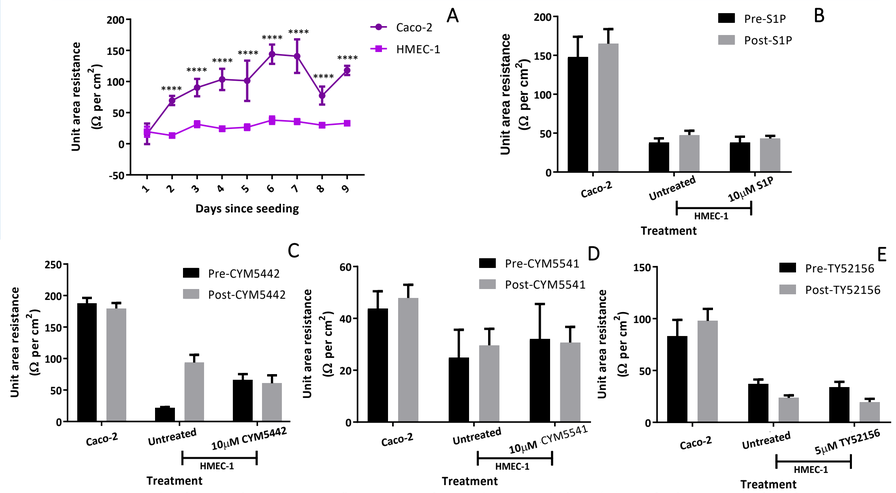


Figure 3. Electrical resistance of Caco-2 and HMEC-1 over 9 days (A), and when treated with S1P (B), CYM5442 (C), CYM5541 (D) and TY52156 (E)

- Caco-2 showed a general trend of significantly higher unit area resistance than HMEC-1 over the course of 9 days ($p<0.0001$). (Number of repeats =3)
- There is no significant difference in the unit area resistance before and after treatments with S1P and the drugs. This is reflected by the overlapping standard error of the mean. (Number of repeats =3)

Conclusion

- HMEC-1 is a good model for blood vessel lining as the cells grow to form a monolayer. This aligns with other published research. Meanwhile, Caco-2 provides a good positive control as the cells has no targets for the molecules of interest and can form multilayers, hence, difficult for the dye to penetrate.
- CYM5442 significantly improved the barrier integrity of the HMEC-1 model
- Caco-2 has a significantly higher unit area resistance than HMEC-1 over the course of 9 days. This agrees with the other experiments that Caco-2 is a better barrier than HMEC-1

Future work

- HMEC-1 only imitated healthy blood vessel lining. Further investigation on damaged lining models is encouraged as they are more similar to the damaged donor organs.
- There are findings in this research group that have shown the presence of targets for S1P and its related drugs in kidney tissues. The study on the effects of S1P and its related drugs can be investigated further in donor organs by adding into the perfusion.

References: (1.) Levkau B. Circ Res. 2008; 103(3):231-233 (2.) Blaho V, Hla T. Journal of Lipid Research. 2014; 55:1596-1608