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Compatibility of Caco-2 cells with Simulated Intestinal Fluid-Predicting Permeability

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SUMMARY

The Caco-2 cell model is considered the gold standard when analysing drug permeability and risk-assessing bioavailability. The use of Simulated Intestinal Fluid (SIF) as a tool in the Caco-2 models is beneficial as this generates a more physiologically relevant model. Existing work using biorelevant media in Caco-2 cells has not fully captured the large-scale variability that can exist within the GI fluids. Furthermore, there is some evidence that the composition of biorelevant fluids can disrupt the Caco-2 monolayer, particularly when high levels of bile and cholesterol are present in these media. Therefore, understanding the compatibility of the simulated intestinal media with the Caco-2 cells is essential to determine its use as transport media in a Caco-2 assay.

INTRODUCTION

Caco-2 cells under normal culture conditions have the ability to spontaneously differentiate into monolayers which are joined through intracellular tight junctions. Along with this the cells can express transporters on their membrane, which play a vital role in the drug absorption across the intestinal membrane. The Caco-2 model is one of the most accepted models of predicting drug permeability following oral absorption (Ingels, *et.al*, 2002). The FDA biowaivers M9 guideline in 2021 states "The results from Caco-2 permeability assays should be discussed in the context of available data on human pharmacokinetics.".

Currently the Caco-2 model typically employs the solubility of the drug in Hanks balanced salt solution before measuring the permeation across the monolayer, however this fails to mimic the in vitro conditions.

MATERIALS AND METHODS

Caco-2 cells from American Type Culture Collection (ATCC) were donated by Dr. Louise Young of the University of Strathclyde. They were grown at 37°C with 5%CO_2 with the culture media components being Dulbecco's Modified Eagle's Medium (DMEM) with L-glutamine without sodium pyruvate supplemented with 10% Foetal calf serum, 1% non-essential amino acid (100x), 1% PEST (Penicillin 10,000 U ml^{-1}-Strepomycin 10,000 U ml^{-1}). All culture reagents were bought from Fisher scientific (Life Technologies, Paisley). Cells were used between passage 16-25.

Compatibility studies were performed in triplicate with cells seeded at a density of 10,000 for each well in 96 well plate. Following a 3-day incubation to allow cells to become confluent the cells were exposed to different compositions of simulated intestinal media. The Abuhassan and Riethorst

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FaSSIF media compositions were based off Human intestinal samples (Abuhassan, *et.al* 2022 and Riethorst *et.al*, 2016) (table 1). The cells were exposed to media for 2 hours prior to 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay being conducted based on manufactures protocol (Promega, UK, Cat no. G4000).

Table 1. *Composition of each media point (mM).*

Media	BS (mM)	PL mM)	FFA mM)	CL mM)	[pHxTAC]
HBSS	-	-	-	-	-
Bio Relevant	3	0.75	-	-	-
Abuhassan -1	1.06	0.16	1.04	0.01	15.07
Abuhassan -2	11.45	2.48	2.88	0.38	122.4
Abuhassan -9	3.46	0.52	1.64	0.032	36.96
Riethorst -1	1.6	0.17	0.07	0.04	5.54
Riethorst -2	3.1	0.39	1.69	0.08	41.63
Riethorst 9	36.18	5.78	15.03	0.2	458.05

1=minimum, 2=maximum, 9=median, BS = Bile salt, PL= Phospholipid, FFA=Free fatty acid CL= Cholesterol, TAC= Total amphiphile concentration.

RESULTS AND DISCUSSION

The absorbance reading at 570nm was taken and the cell viability calculated relating to percentage viability against HBSS, as a control. Data shown in figure 1.

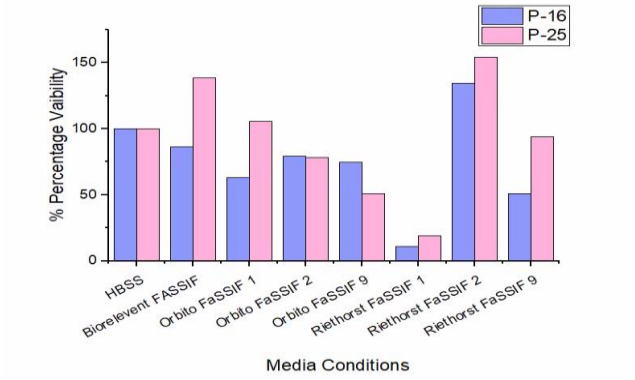

Figure 1. Percentage of cell viability of Caco-2 in relation to HBSS

Understanding the media components and the role this has on the cell integrity can be beneficial as it allows screening of media prior to the addition to the monolayers. In the fasted state media, there was similar cell survival to what was seen with HBSS. This shows that there is possibility to substitute the HBSS in current assay to more biorelevant media. Most of the FaSSIF medias resulted in similar or greater survival to those cells incubated in HBSS except for Riethorst FaSSIF 1, which has a pH of 2.4. This acid media is likely to be responsible for the low viability observed. Both Abuhassan and Riethorst medias were based off a sample set collected from a 20-patient, Riethorst media minimum and maximum take into consideration the actual values from the volunteers. Whereas Abuhassan media although taken from the same sample set media values are representative values statistically generated for low, centre, and high points of the volunteer population.

The medias with high BS, PL, FFA or CL values were anticipated with to have a higher toxicity to the cells, yet this was not always observed. Elevated bile salt levels act as detergents on lipids, promoting oxidation and the generation of reactive oxygen species (ROS), which in turn increase cellular toxicity. PL, FFA and CL can interact with cell membranes which may also reduce viability. Therefore, investigating the colloidal structures formed by BS, PL, FFA and CL and their interactions with Caco-2 cell membranes is critical to understanding their impact on cell viability.

Permeability assays are performed using cells at high passages due to the formation of tight junctions. However, in the absence of tight junctions, cells at higher passages (p-25) demonstrate greater survival in the simulated intestinal media compared to cells at lower passages (p-16).

CONCLUSIONS

This study highlights an opportunity to use a more biologically relevant media in Caco-2 drug permeability studies.

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