Innovative Microphysiological Model Using HepatoXcell™ Primary Human Hepatocytes and Locsense Artemis

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1. Introduction

The development of a highly representative in vitro gut-liver model is essential for improving drug testing accuracy while minimizing the reliance on animal studies. Traditional animal models often fail to fully replicate human physiology, leading to discrepancies in drug metabolism and toxicity predictions.[1] A physiologically relevant gut-liver system would provide a more accurate platform to study nutrient absorption, drug bioavailability, and toxicity, ultimately enhancing preclinical research. This approach aligns with the 3Rs principle (Replacement, Reduction, and Refinement), promoting ethical and sustainable alternatives to animal testing while advancing biomedical research and drug development.

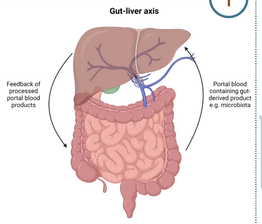


Figure 1: Gut-liver axis interaction.

2. Background

The gut-liver axis represents a bidirectional communication network between the gastrointestinal (GI) tract and the liver, playing a pivotal role in metabolism, immunity, and detoxification. This connection is primarily facilitated by the portal vein, which transports nutrients, metabolites, microbial products, and toxins from the gut to the liver for processing. To develop a highly representative model, the selection of cellular components is critical. Caco-2 cells are ideal candidates for an in vitro intestinal barrier model as they spontaneously differentiate into polarized, enterocyte-like cells, forming tight junctions that mimic the intestinal epithelium. HepatoXcell primary human hepatocytes are excellent candidates for an in vitro liver model due to their retention of key liver-specific functions. These cells can provide early insights into drug behavior in the human body, potentially reducing the risk of adverse effects in clinical trials. **Figure 2** illustrates the morphology and fluorescent expression of key markers in the primary hepatocytes.

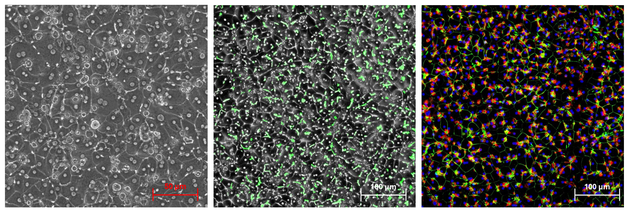


Figure 2: HepatoXcell. (A) Phase contrast image (20x) of HepatoXcell Pro cells plated in collagen I-coated plates in sandwich culture. (B) HepatoXcell Pro cells imaged at 10x magnification following staining with 5(6)-Carboxyfluorescein diacetate (CDFDA), demonstrating bile accumulation in the bile ducts (green). (C) HepatoXcell Pro cells on day 5 of culture were imaged at 10x magnification under a fluorescent microscope following staining with E-Cadherin (green) and Occludin (red) antibodies, illustrating cell-cell adhesion and epithelial tight junctions, respectively. The nuclei were counterstained with DAPI (blue).

3. Aims

Development of a highly representative in vitro gut-liver model using Caco-2 gut epithelial cells and HepatoXcell primary human hepatocytes to reduce the burden of animal testing

4. Methods

The gut-liver axis was composed of Caco-2 gut intestinal cells (ATCC HTB-37™) established on a transwell membrane in DMEM medium and HepatoXcell Pro primary human hepatocytes (ATCC PCS-450-011™) plated on collagen I coated wells in HepatoXcell maintenance medium (**Figure 3**).

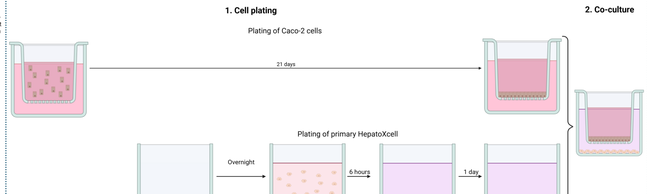


Figure 3: Schematic representation of plating. Caco-2 cells were cultured on Transwell membranes for 3 weeks. Primary hepatocytes were cultured on collagen-I coated well plates. After successful plating, cells are combined to form a co-culture.

To thoroughly analyze the capabilities and representative characteristics of this model, we measured barrier integrity and viability under stress conditions. A schematic representation of the experimental setup is illustrated in **Figure 4**.

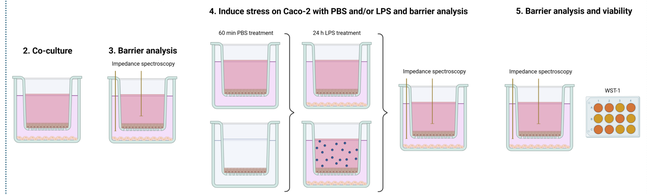


Figure 4: Schematic representation of methods. On Day 1, Caco-2 cells and hepatocytes were cultured as previously described. On Day 2, the cells were combined to establish a co-culture. On Day 3, barrier integrity was assessed using impedance spectroscopy with the Artemis ST of Locsense. On Day 4, stress was induced by washing with PBS and/or incubation with LPS (2 µg/mL), followed by barrier integrity analysis using impedance spectroscopy. On Day 5, the viability of Caco-2 cells and primary hepatocytes was measured using the WST-1 assay, and barrier integrity was again analyzed using impedance spectroscopy.

The Artemis ST by Locsense offers precise measurements of electrical impedance and phase data. Using circuit model fitting (CMF), the impedance data obtained with the Artemis is fitted to an equivalent electrical circuit model for the cell culture. This process provides the values and uncertainties for the various elements of the electrical circuit, including the cell culture, medium, and electrodes. From these values, the transepithelial electrical resistance (TEER) can be extracted. The workflow is illustrated in **Figure 5**.

Figure 5: Schematic overview of impedance set up. The smart lid fit a 12-well plate and can measure the impedance and phase data of 12 wells in several minutes. These data are transferred to the laptop which performs CMF to extract TEER data.

5. Results

The most important requirement for a co-culture is symbiosis; both cell types need to survive each other and each other's medium. Therefore, we used WST-1 to analyze the viability of Caco-2 cells in hepatocyte medium and the viability of the primary hepatocytes with and without Caco-2 transwell cultures. Results are depicted in **Figure 6**. Both Caco-2 cells as well as the hepatocytes do not show any significant change in viability due to the co-culture. We can state that this co-culture is viable.

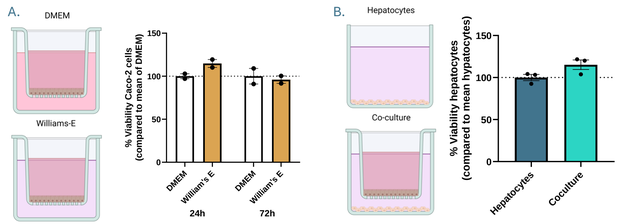


Figure 6. Viability WST-1. (A) Relative viability of Caco-2 cells in basolateral hepatocyte maintenance medium as compared to Caco-2 cells in basolateral DMEM medium after 24 and 72 h. (B) Relative viability of hepatocytes in co-culture with Caco-2 cells compared to hepatocytes in mono-culture.

The objective of this research is to thoroughly investigate the tolerance of the hepatocyte Caco-2 co-culture model by inducing stress on the Caco-2 intestinal barrier and analyzing its barrier integrity and asses the viability of both cell types in the co-culture.
Various stressors elicit distinct physiological responses. While thermal stress and hypoxia reduce transepithelial electrical resistance (TEER),[2] shear stress enhances TEER.[3] In this study, we induced mild shear stress via phosphate-buffered saline (PBS) washing and applied chemical stress using 2 µg/mL lipopolysaccharide (LPS). As shown in **Figure 7A**, PBS washing increased TEER, whereas LPS did not alter it, indicating a robust response of the co-culture system. Furthermore, although barrier integrity responded to the induced stress, the viability of Caco-2 cells remained unaffected.
Moreover, the integrity of the barrier is further highlighted by the unaltered hepatocyte viability. Despite the potential of LPS to induce hepatocyte cell death, a robust intestinal cell barrier should prevent LPS translocation to the basolateral side, thereby mitigating hepatotoxicity. As illustrated in **Figure 7C**, hepatocytes exhibit no significant differences in viability, underscoring the protective function of the intestinal barrier.

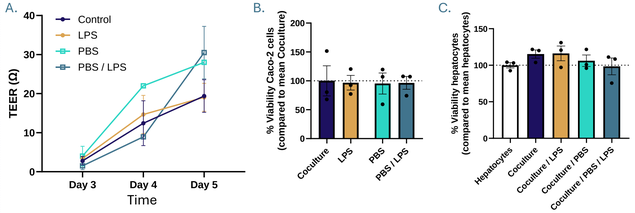
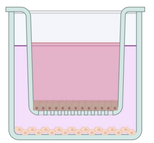


Figure 7. TEER and viability WST-1. (A) TEER over time of the Caco-2 intestinal barrier. (B) Relative viability of Caco-2 cells under PBS and/or 2 µg/mL LPS-induced stress compared to Caco-2 cells in control medium. (C) Relative viability of hepatocytes in co-culture with Caco-2 cells under stress compared to hepatocytes in mono-culture.

6. Conclusions

In this study, we developed a highly representative in vitro gut-liver model using Caco-2 gut epithelial cells and HepatoXcell Pro primary human hepatocytes. These two cell types can be co-cultured without compromising each other's viability and behavior. Stress-induced changes in barrier function do not affect the viability of Caco-2 cells. Our model demonstrates that a healthy gut barrier protects primary hepatocytes from cytotoxic compounds. This model enhances our understanding of the gut-liver axis and improves preclinical drug development while adhering to the 3R principle, thereby reducing the reliance on animal testing.

7. Take home message



- We have developed a highly representative gut-liver axis model utilizing ATCC's HepatoXcell Pro Primary Human Hepatocytes and Caco-2 intestinal cell lines.
- The Artemis ST by Locsense is an effective tool to measure electrical impedance and membrane barrier function.
- The Caco-2 transwell intestinal barrier demonstrates substantial resistance to LPS-induced chemical stress, resulting in the preservation of hepatic culture integrity.

8. References

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8. Author information