Integration of Incucyte® Analytics and Multi-Organ ToxPlate (Kidney, Small Intestine, and Liver) for NAMS-Based Hazard and Risk Assessment of Compounds

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Abstract

Background and Purpose: Advanced primary cell-based 3D human tissue models are key components of New Approach Methodologies (NAMs), providing a reliable alternative to animal testing for hazard and risk assessment of chemicals, pharmaceuticals, and formulations. To overcome the limitations of traditional single-organ systems in predicting human responses, we developed a 3D human Multi-Organ ToxPlate (MOTP) that integrates small intestine, liver, and kidney tissue models within a 96-well plate format. This platform was adjusted to be compatible with Sartorius's Incucyte® system for high-throughput imaging and automated live-cell analysis, enabling dose-range finding experiments for single or multiple drugs across three organ systems possible simultaneously. This integrated workflow reduces variability, accelerates timelines, lowers cost, and provides real-time imaging with automated EC50 calculations as an endpoint.

Methods: Tissue Preparation: Cells representing three organ systems (liver, kidney, and intestine) were seeded into a 96-well ToxPlate, with each organ system allocated 32 wells. Cultures were maintained under submerged conditions for 1–3 days, followed by air-liquid interface (ALI) conditions for 12 days to achieve proper differentiation and stratification. To validate the workflow, we first reconstructed a single-organ plate of the 3D human kidney model and dosed it with Cisplatin (known nephrotoxicant) and Oxaliplatin (non-toxicant) at various concentrations. Cell death was monitored using Incucyte® Cytotox Green reagent (Sartorius, Ann Arbor, MI) dosed with the test compound. Next, we developed a 96-well multi-organ ToxPlate (MOTP) incorporating 3D tissues of intestine, liver, and kidney. Drug-exposed tissues were incubated at 37°C and imaged in an Incucyte® S3 Live-Cell Analysis System housed within the incubator. Green fluorescence images were acquired at 10× magnification every 4 hours for 48 hours. Global mean fluorescence values were calculated using the Basic Analyzer module of the Incucyte® software, where higher fluorescence indicates greater cell death. Since Incucyte® imaging and analysis is non-invasive, TEER, MTT viability, and histological measurements were performed after Incucyte® data collection.

Results: The study demonstrated a dose-dependent decrease in TEER following Cisplatin treatment of the 3D kidney tissue model. Incucyte® live-cell analysis revealed EC50 values for Cisplatin ranging from 11 to 25 µM, consistent with previously reported manual measurements. Then, a 48-hour, seven-dose range finding study was performed using Cisplatin and Oxaliplatin on ToxPlate containing kidney tissues integrated with the Incucyte® workflow. Cisplatin exposure resulted in a clear dose-dependent reduction in TEER and MTT viability. Incucyte® analytics also revealed a difference in drug response for Cisplatin (17 µM) compared to Oxaliplatin (88 µM) in human 3D kidney tissue model. We further expanded the study to assess the toxicity potential of three drugs Cisplatin, Fialuridine, and Troglitazone on the ToxPlate containing a) EpiKidney and EpiIntestinal and b) EpiKidney, EpiIntestinal, and EpiLiver tissue models. Post-treatment TEER measurements indicated that Cisplatin was toxic to all three tissue models at higher doses. Fialuridine, a known human liver toxicant, exhibited selective hepatotoxicity at a concentration of 200 uM without affecting kidney or intestinal tissues. Troglitazone showed toxicity toward liver and kidney at higher concentrations, while its effect on intestinal tissues was variable.

Conclusions: The integrated MOTP–Incucyte® workflow provides a robust and economical platform for drug toxicity testing. This trio-tox assay system is physiological and accelerates *in vitro* toxicity predictions and supports early detection of organ-specific and off-target effects. This approach reduces reliance on animals and helps mitigate late-stage clinical failures.

Methods

Tissue preparation: Cells representing three organ systems (liver, kidney and intestinal) were seeded into a 96-well tox plate with each organ system allocated 32 wells. The cells were initially cultured under submerged conditions (1-3 days) followed by air-liquid interface (ALI) culture condition for 12 days to proper differentiation and stratification (Figure 1).

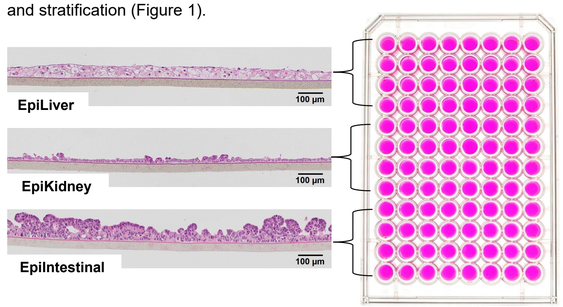

Figure 1: Representation of a multi-organ tox plate (MOTP) containing intestine, liver, and kidney tissue models in a single 96-well plate format.

Methods (cont.)

Integrated workflow: To test the integrated workflow, we first reconstructed a single organ plate of the 3D human kidney model and dosed with Cisplatin (kidney toxicant) and Oxaliplatin (non-toxicant) at various concentrations The Incucyte® Cytotox Green reagent (Sartorius, Ann Arbor, MI) was used to monitor cell death. Next, we expanded the study and developed a 96-well muliti-organ tox plate (MOTP) reconstructing 3D tissue of intestine, liver, and kidney on the same tox plate. The drug exposed tissues were then incubated at 37°C and imaged in an Incucyte® S3 Live-Cell Analysis System (Sartorius, Ann Arbor, MI) housed within the incubator (Figure 2). Green fluorescence images were acquired at 10X magnification in real-time every 4 hours for a total of 48 hours. For each image, global mean fluorescence values were calculated using the Basic Analyzer module of the Incucyte® software (Figs 3-5) . A higher observed mean fluorescence value would correspond to higher cell death.

Tissue barrier integrity: To qualify tissues for drug testing, a pre-dose transepithelial electrical resistance (TEER) measurement was performed on all tissues on the tox plate. Post drug exposure TEER measurement was also performed in all experiments Fig 6 &7).

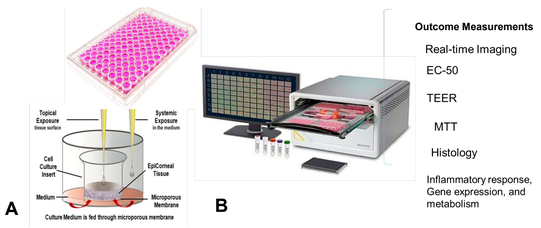

Figure 2: Schematic representation of the reconstruction of the 3D human tissue model (A) and integration with high content imager (Sartorius Incucyte S3) (B). In this Integrated workflow, tissues can be dosed with drugs and placed in the Incucyte® chambers to allow for comprehensive analysis of drug effects on human tissues in real-time and to calculate EC-50 values. Additional outcome measurements such as TEER, MTT, histology, gene expression, and inflammatory responses can be performed.

Results

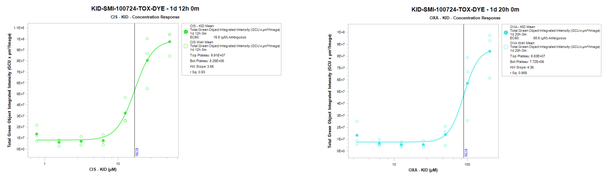

Figure 3: EC50 value of Cisplatin and Oxaliplatin following a 48-hour exposure of the 3D human kidney tissue model using Incucyte® software module. The Calculated EC 50 value for cisplatin and Oxaliplatin was found to be 17.1 µM. And 88µM, respectively.

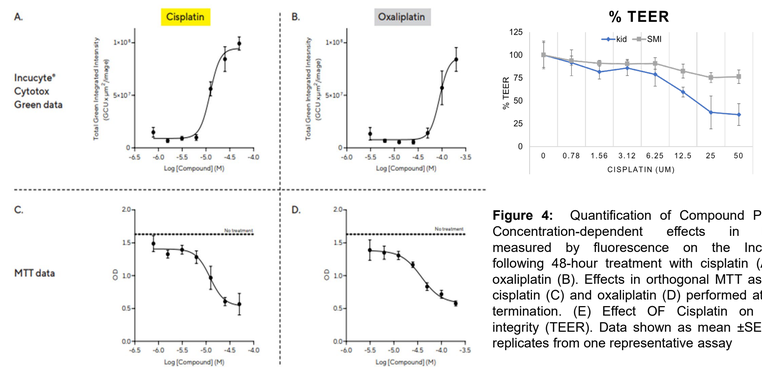

Figure 4: Quantification of Compound Potency. Concentration-dependent effects in toxicity measured by fluorescence on the Incucyte® following 48-hour treatment with cisplatin (A) and oxaliplatin (B). Effects in orthogonal MTT assay for cisplatin (C) and oxaliplatin (D) performed at assay termination. (E) Effect OF Cisplatin on barrier integrity (TEER). Data shown as mean ±SEM of 3 replicates from one representative assay

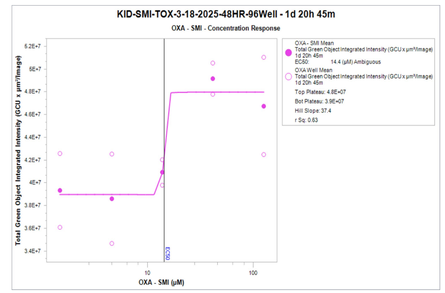

Figure 5: EpiIntestinal tissues were exposed to different concentrations the Oxaliplatin for 40 hr in the presence of CytoTox green dye (cell death marker). EC50 values were calculated (88.6uM) with Incucyte® digital solution software. Note: Oxaliplatin is a colorectal cancer drug that has effect on cellular division and proliferation.

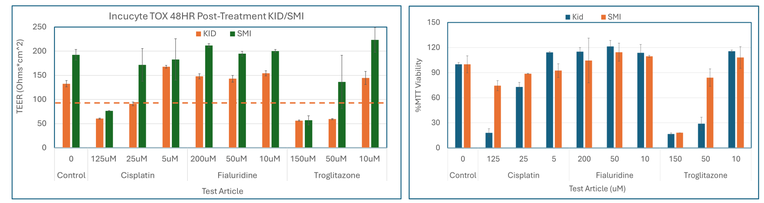

Figure 6: ToxPlate containing EpKidney and intestinal tissues. Tissues were exposed to different concentrations of drugs for 40 hr in the presence of Cytotox green.EC50 values were calculated with Incucyte software. At the end, membrane integrity measurement was performed and % TEER was calculated

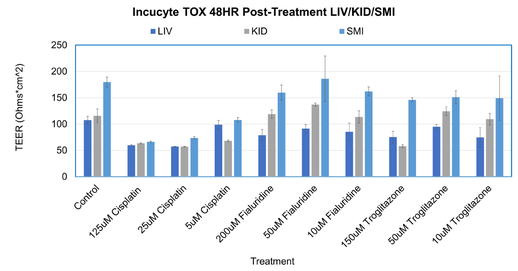

Figure 7: ToxPlate containing EpiLiver, EpiKidney and EpiIntestinal tissues: Barrier integrity measurement (TEER) of the 3D human Trio-Tox plate containing liver, kidney, and small intestinal tissues was performed following 48-hour exposure to different concentrations of three test drugs.

Conclusion

- Reconstructed advanced 3D human tissue models possess structural architecture similar to their *in vivo* counterparts (**Fig 1**).
- The integrated workflow allows high content imaging and dose response analysis in real time (Figs 2 - 5).
- Development of Multi-Organ Tox Plate of 3D human intestinal, kidney, and liver tissues integrated with a an Incucyte® workflow to collect data in real time is a novel approach in drug toxicity studies (Figs 3-7).
- Exposure of liver, kidney, and intestinal tissue models to Fialuridine shows organ specific (liver) toxicity that mimics a human response (**Fig 7**).
- Our data suggest that integrating 3D tissue models with the Incucyte workflow and Cytotox Green Dye could be a promising approach for studying drug toxicity in an *in vitro* microenvironment..