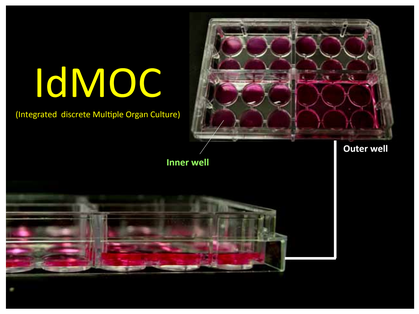
The Integrated Discrete Multiple Organ (IdMOC™) Technology as an In Vitro Model For Systems Toxicology

Albert P. Li, PhD
In Vitro ADMET Laboratories, 9221 Rumsey Road, Columbia, MD 21045

Contact Information:
Aarti Uzgare, PhD
auzgare@apsciences.com

INTRODUCTION

A major limitation of in vitro toxicity testing is the use of single cell-type cultures for the evaluation of organ-specific toxicity. Such systems ignore the critical interactions between different cell-types within an organ or between multiple organs. As a result, current models do not adequately mimic in vivo toxicological data. The Integrated Discrete Multiple Organ Co-culture (IdMOC™) technology was developed in our laboratory to overcome this major deficiency.



In the IdMOC™ plate, multiple cell types are seeded in inner wells and incubated for 24 hours to allow attachment, after which the larger, rectangular, outer well is flooded with media containing substrates or test compounds. The flooding medium permits interconnection of multiple inner wells mimicking the integration of multiple organs via the systemic circulation.

CHARACTERIZATION AND VALIDATION

Cell Growth Comparison of 3T3-L1 Cells in IdMOC™ Versus Standard Plates

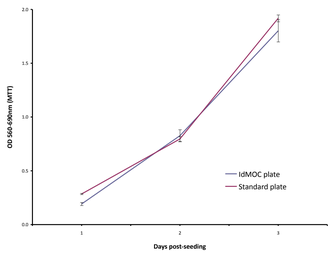


Figure 1A. Growth curves of 3T3-L1 cultures seeded in regular 24-well (BD FalconTM, Catalog # 353226) and IdMOC™ plates (IVAL, LLC, catalog # 71035). Cells were plated (8,000/well) on day 1 in a volume of 200ul (IdMOC™ plate) or 500ul (standard plate) DMEM. The next day IdMOC™ plates were flooded with 4 ml of media per chamber while standard plates received 500ul of fresh media. MTT assay was performed on days 2, 3 and 4 using standard protocols. Results obtained are expressed as mean OD values ±sem of n=12 (day 2) and n=18 (day 3 and 4) samples.

IdMOC™ Chamber-to-Chamber Variability

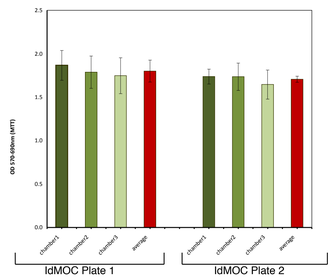


Figure 1B. MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] was performed on 3T3-LI cells plated (8,000/well) in different 24-well IdMOC™ plates for a period of 3 days. Briefly, 0.5mg/ml MTT was added for a period of 4 hours followed by solubilization in 200ul of 70% iso-propanol for 1 hour. The entire 200ul was transferred to a 96 well plate and read in a multi-plate reader at 560 nm using a background subtraction at 690nm. Results expressed are the mean OD values obtained at 570 nm (minus 690nm) for each outer chamber (n=6/chamber) ±sem.

ORGAN-SPECIFIC TOXICITY

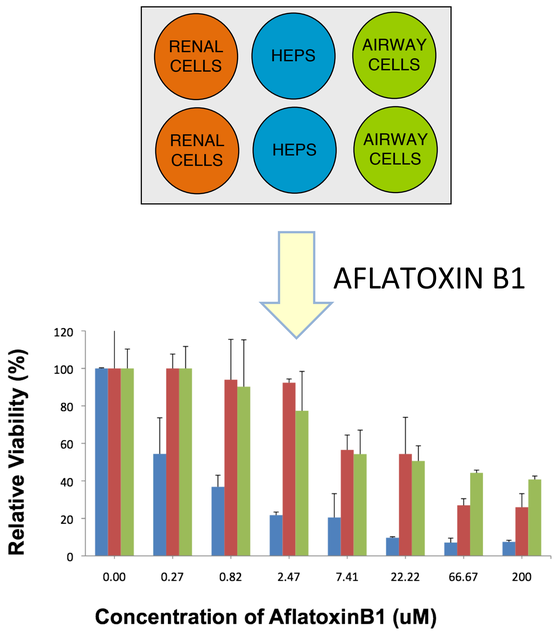


Figure 2A. AflatoxinB1 is selectively cytotoxic to hepatocytes. Human renal proximal tubule epithelial cells, human hepatocytes and human small airway epithelial cells were cultured in IdMOC™ plates (24-well) and treated with different concentrations (3-fold increases) of AflatoxinB1 for 48 hours. Results are shown as relative viability (mean±sd, n=3) based on cellular ATP content. Selective toxicity of AflatoxinB1 towards hepatocytes is evident at concentrations ranging from 0.82-200uM.

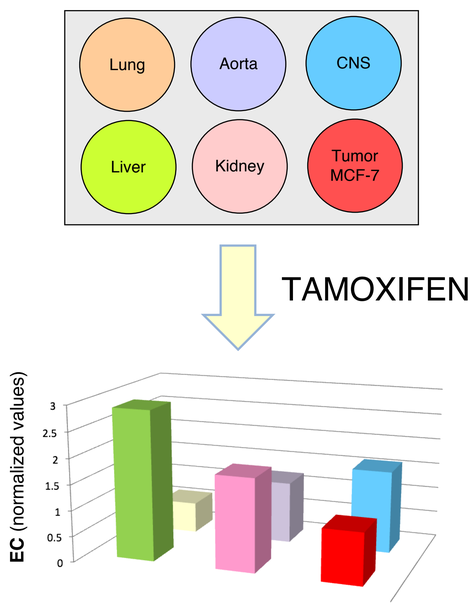


Figure 2B. Tamoxifen is more cytotoxic to metastatic breast carcinoma cells than most normal primary human cells. Human small airway epithelial cells (lung), human aortic endothelial cells (HAEC: aorta), human astrocytes (nervous system), human hepatocytes, renal proximal tubule epithelial cells, and human breast cancer cells (MCF-7) were cultured in IdMOC plates (24-well) and treated with different concentrations of tamoxifen for 48 hours. The effective concentrations at which 50% of cell death occurred was calculated, i.e. EC50, based on cellular ATP content and normalized to the values obtained from MCF-7 cells (n=3 samples per concentration tested).

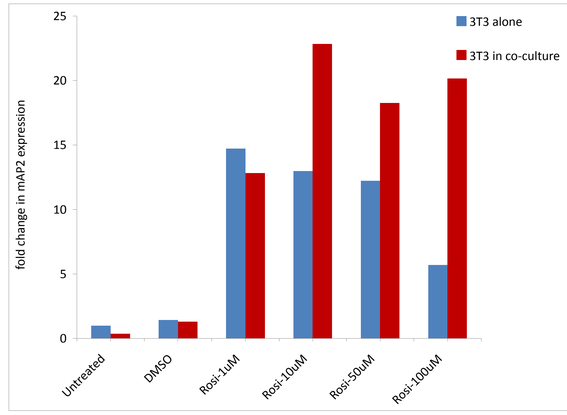


Figure 3. Hepatocytes promote adipogenic differentiation of 3T3-L1 cells. 3T3-L1 cells (75,000 cells/well) were plated in the presence and absence hepatocytes (250,000 cells/well) and Matrigel was added at a final concentration of 0.25mg/ml. Cells were further treated with a differentiation cocktail containing 3nM insulin, 0.25uM dexamethasone and 500uM IBMX either in the presence of DMSO (vehicle control) or Rosiglitazone (1-100uM). Cells were lysed 24 hrs later and RNA was extracted, reverse transcribed and quantified for the expression of mouse AP2 (adipogenic marker) using qPCR. Results were normalized to housekeeping gene-M36B4 and are shown as fold change over untreated controls, determined by the ΔΔCt method.

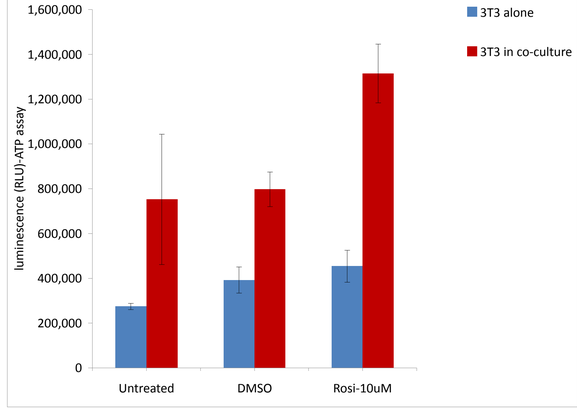


Figure 4. Hepatocytes promote proliferation of 3T3-L1 cells. 3T3-L1 cells (75,000 cells/well) were plated in the presence and absence hepatocytes (250,000 cells/well) and Matrigel was added at a final concentration of 0.25mg/ml. Cells were further treated with a differentiation cocktail containing 3nM insulin, 0.25uM dexamethasone and 500uM IBMX either in the presence of DMSO or 10uM Rosiglitazone. Cells were lysed 5 days later and cellular ATP content was quantified as relative luciferase units (RLU). Results shown are mean±sem of n=3 samples.

CONCLUSIONS

IdMOC™ technology can be used to evaluate

- **Multi-organ toxicity**
- **Effectiveness of therapeutic drugs**
- **Influence of one cell-type over the other (paracrine signaling)**
- **Potential replacement of animal studies with in vitro cell culture methods**