Organoid Therapeutics: Organoid Enzyme Fluorometric Assay

I. Chickanosky, J. Krempa, T. Pierre, S. Shah, M. Van Buskirk, A. Vesco

Biomedical Engineering, Materials Science Engineering, Chemical Engineering, Mechanical Engineering

Background

Needs Statement

- An improved drug screening method to increase reliability and accuracy of pharmacological testing by using drug-induced liver injury (DILI) as a metric of damage
  - *Problem:* 44,000 Americans annually experience hepatotoxic drug effects that lead to DILI[1] and 10% of cases lead to liver transplantation or death[2]
  - *Population*: Pharmaceutical companies
  - *Outcome:* Reliable and affordable liver organoid fluorometric assay to reduce DILI

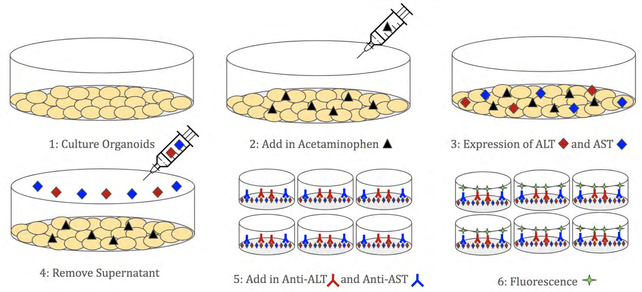
Status Quo

- Current standard to evaluate drug toxicity is animal testing
  - LD50 Test: Lethal dose is dose needed to kill half of animal population

Gap

- Current tests are expensive ($3,050 per test) and do not translate to human settings as well[3]

Proposed Solution



Organoid and 2D Hepatocyte Enzyme Fluorometric Assay

- Generate a FLISA standard curve
- Culture liver organoids and 2D hepatocytes
- Expose liver organoids to experimental drugs
- Recognize cell viability with a live dead assay
- Analyze aspartate transaminase and alanine aminotransferase levels (enzymes indicative of DILI) with a FLISA assay
- Correlate enzyme levels to cell viability

Results

Liver Organoid Culture

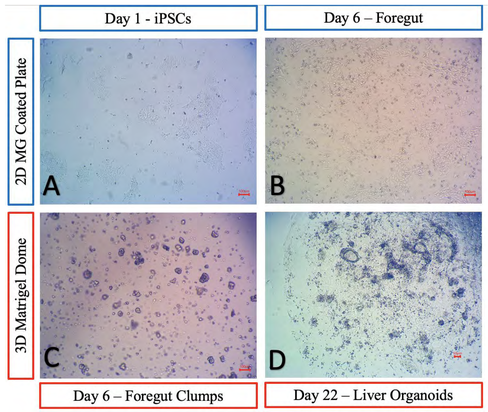


Figure 1. Differentiation Stages from iPSC to HLO in 22 Days (Blue: Matrigel Coated Plates and Red: 3D Matrigel Domes)

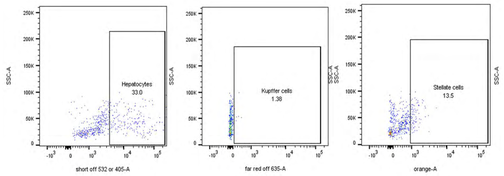


Figure 2. Dot Plot of Fluorescence Intensity vs. Side Scatter Intensity for Hepatocytes, Stellate, and Kupffer Markers

Standard Curve

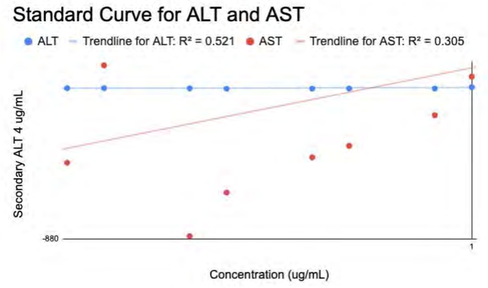


Figure 3. ALT and AST Standard Curve

Live Dead Assay

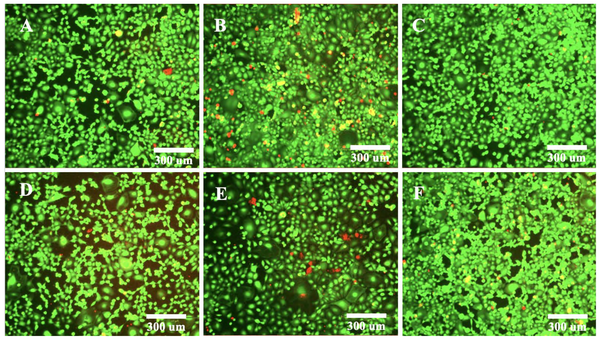


Figure 4. Live Dead Assay with Concentrations of Acetaminophen: 0 mM (A), 0.5 mM (B), 1.0 mM (C), 2.0 mM (D), 5.0 mM (E), and 10.0 mM (F)

Optimization for Wavelength

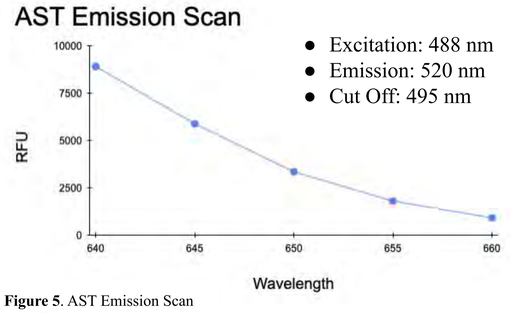


Figure 5. AST Emission Scan

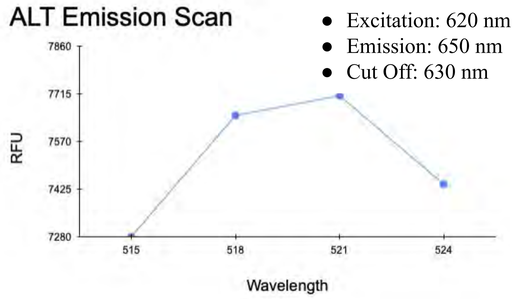


Figure 6. ALT Emission Scan

Conclusions

Human Liver Organoid Culture

- Successfully cultured organoids with 30-60% hepatocytes, 15-30% stellate, and 0-2% kupffer cells (similar to literature values)
- Unable to expand organoids
- Future directions: expand organoids and run live dead and FLISA assay with them

Live Dead Assay

- Following cell viabilities for drug concentrations: 96.1% (0 mM), 85.2% (0.5 mM), 90.7% (1.0 mM), 97.8% (2.0 mM), 94.3% (5 mM), 86.8% (10 mM)
- Inconclusive results
- Future direction: run live dead assay with less confluent cells at around 70% instead of 90%

Standard Curve

- Determined optimal wavelengths for emission and excitation for ALT and AST
- Standard curve R^2 values for ALT (0.521) and AST (0.305) are low
- Future directions: rerun standard curve measurements with new microplate reader

Acknowledgements

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References

[1] David S, Hamilton JP. Drug-induced Liver Injury. US Gastroenterol Hepatol Rev. 2010;6:73-80.
[2] M. Lisi, D., 2020. Drug-Induced Liver Injury: An Overview. [online] Uspharmacist.com.
[3] Meigs, L., Smirnova, L., Rovida, C., Leist M., and Hartung, T. Animal Testing and its Alternatives – the Most Important Omics is Economics. ALTEX; 35(3): 275-305. doi:10.14573/altex.1807041
[4] Dened, Lyn. Biodesign: The Process of Innovating Medical Technologies. 2nd Edition. Cambridge University Press; 2015.