Development of a 3D HepaRG based non-invasive optical imaging platform for pre-clinical hepatotoxicity screening

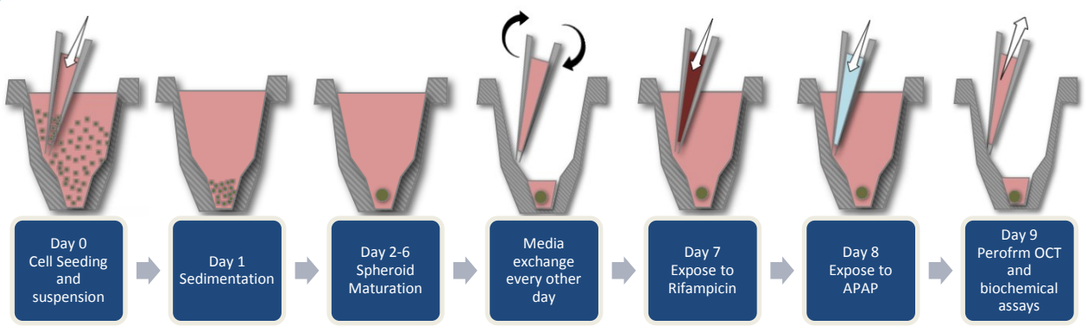
Nicole J Martucci[1,2], Katie Morgan[1], Peter Hayes[1], John Plevris[1], Leonard Nelson[2], Pierre O. Bagnaninchi[2]

[1]Hepatology Laboratory, Royal Infirmary of Edinburgh, University of Edinburgh, [2]MRC Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, EH16 4SB, Scotland, uK Contact : Nicole.Martucci@ed.ac.uk, Pierre.Bagnaninchi@ed.ac.uk

THE UNIVERSITY of EDINBURGH

Introduction |

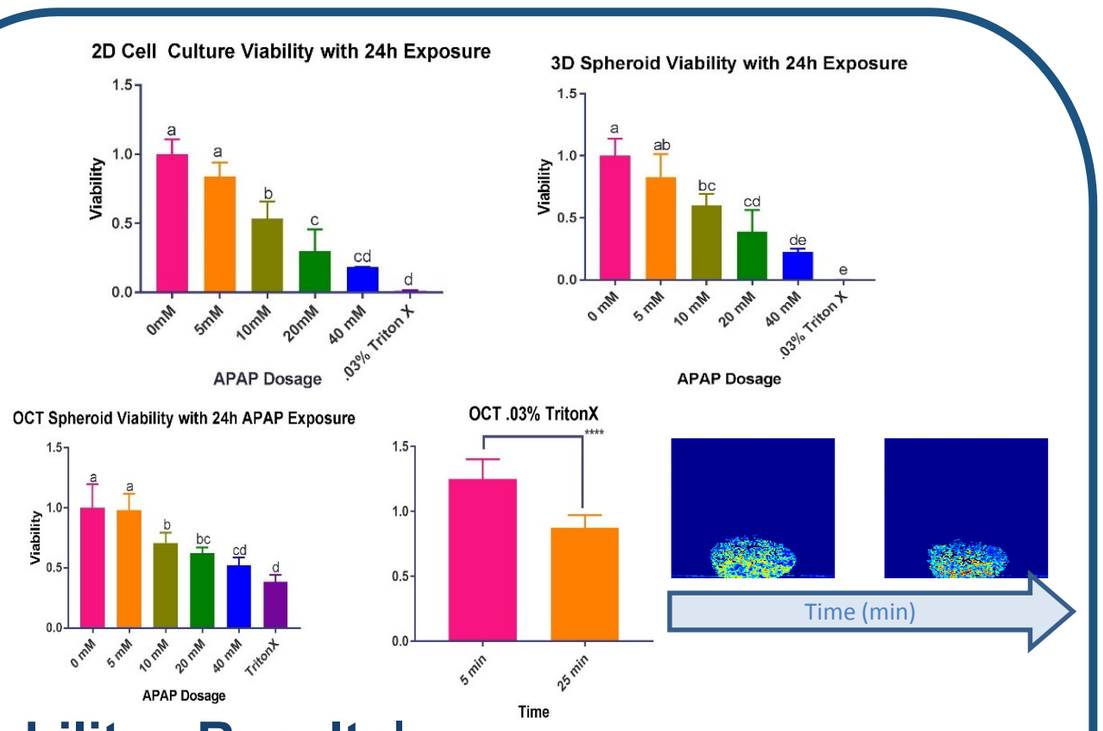
Drug Induced Liver Injury (DILI) accounts for over 50% of the cases of acute liver failure in the USA, and currently there are over 1,000 drugs associated with DILI. DILI is the leading cause of attrition in pre-clinical drug development and withdrawals. Strong incentives exist to create new human-based, *in-vitro liver models to offer better pre-clinical predictions of DILI*



Methods|

Cell Culture: HepaRG cells were cultured using suppliers protocols. Williams E Medium with GlutaMAX™ was used as the basal medium with supplements purchased from Biopredic International. For the 2D culture, cells were seeded at day 0 at 2.4x10 5 cells/cm 2 on a Corning 96 well. For the 3D spheroids, HepaRGs were seeded at day 0 ata concentration of 2,500 cells per well in an 96 well InSphero GravityTrap™ (Perkin Elmer) ULA plate.

Drug Exposure and Viability Testing: Both models were exposed to various doses of paracetamol (APAP) for 24 hours on day 9. OCT was used to monitor 3D spheroids in real time and to quantify cell viability, and correlated with biochemical viability assays. H&E staining was performed on day 14 to verify the presence/absence of a necrotic core in the spheroids.



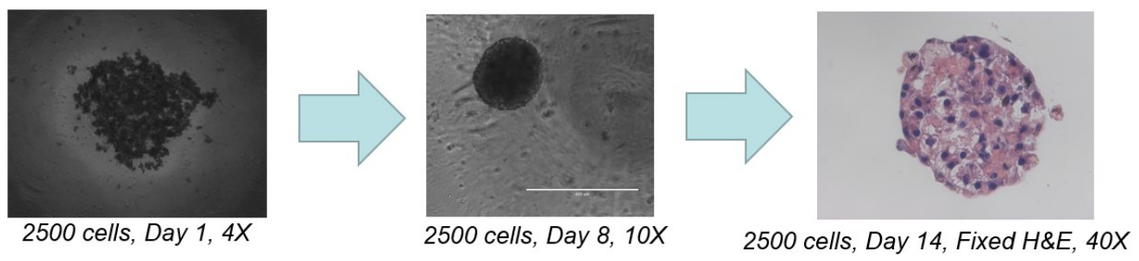
Viability Results|

Biochemical Assay: Cell Titer Glo Luminescent Cell Viability Assay 2D and 3D was used to measure viability of the 2D culture and the spheroids after APAP exposure for 24 hours. Similar trends were seen in both, however, the 2D spheroids were more sensitive to the drug compared to the 3D model, reaching approximately 85% cell death at 40 mM of APAP.

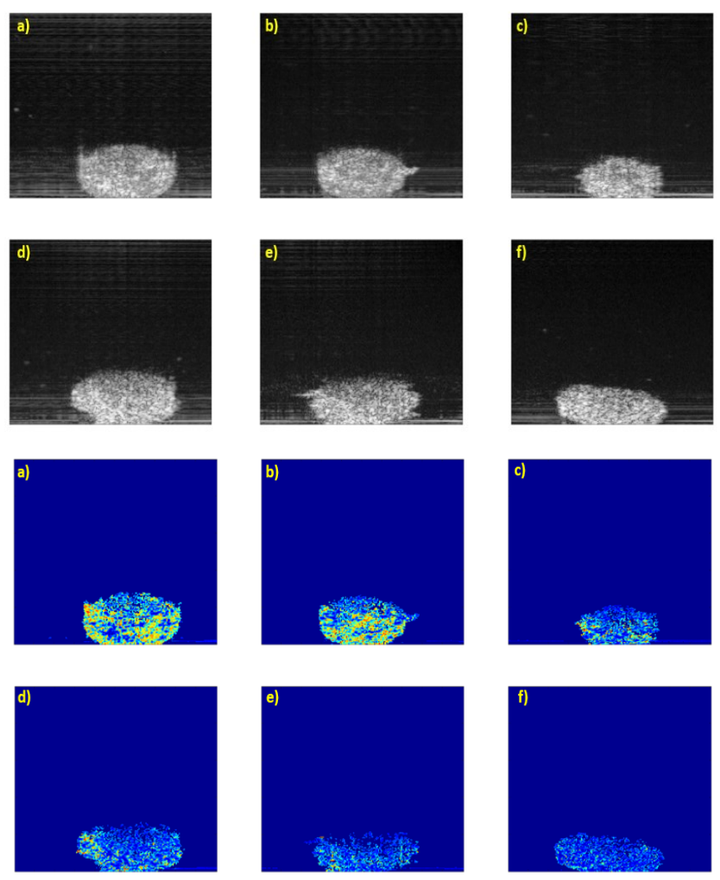
OCT Viability Measurements: OCT was able to measure noninvasively, label free, and in real time the viability of the 3D spheroids with a similar trend as to what the biochemical assays reported.

Spheroidal Formation and Staining|

Images were taken using an EVOS microscope at various magnifications to observe the formation of the spheroids seeded at 2,500 cells per well. Spheroid was approximately 500 um in diameter on Day 1 and 200 um in diameter on both Day 8 and Day 14.
No Necrotic core was seen by day 14



2500 cells, Day 1, 4X — *2500 cells, Day 8, 10X* — *2500 cells, Day 14, Fixed H&E, 40X*



Intensity Images (black and white) correlate to corresponding fluctuation map images (blue).

A) Control (0 mM APAP)
B) 5mM APAP
C) 10 mM APAP
D) 20 mM APAP
E) 40mM APAP
F) 03% Triton-X

Optical Coherance Tomography|

OCT was able to image at a microstructural level 3D liver spheroids in a 1mm x1mm (xz) field of view. No changes that could have been induced by drug toxicity was consistently observed in intensity images. Phase fluctuations were displayed on a colormap. A clear decrease in optical fluctuations associated in a dose-dependent manner can be observed in the fluctuation maps. This demonstrate the ability of the OCT-based toxicity assay to give real-time information on the cell viability, and its ability to spatially resolve cell viability as opposed to conventional biochemical assay.

Conclusion|

Microstructural information and cell viability were retrieved from functional OCT measurements and integrated to investigate acetaminophen cytotoxicity on 3D liver spheroids with good correlation with standard biochemical assays. In this study, we introduced and demonstrated a novel toxicity assay based on optical coherence phase tomography. This new method has the potential to assess noninvasively and label-free drug toxicity in 3D tissue models.

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