Institution: Liverpool John Moores University (LJMU)		
Unit of Assessment: UOA11		
Title of case study: Improving Drug Safety Screening: 3D – Liver Spheroid Toxicity Model		
Period when the underpinning research was undertaken: 2013-2017		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Dr Steve Webb	Reader in Applied Mathematics	01/10/2015 to 16/02/2020
Period when the claimed impact occurred: 2015-2017		
Is this case study continued from a case study submitted in 2014? N		

1. Summary of the impact

Drug-induced liver injury represents a major global health concern and is one of the most common adverse drug reactions. However, standard 2D-monolayer cell culture techniques to capture key aspects of liver physiology can result in underestimation of liver injury as a function of drug exposure. Using mathematical modelling and experimental data, Dr. Steve Webb developed a 3D-liver spheroid model that is more representative of the *in vivo* system. AstraZeneca have since used this system to screen 5000 compounds, impacting the choice of safety molecules to progress with regards to hepatic safety at the discovery phase in a global pharma company.

2. Underpinning research

As part of the NC3Rs 2011 CRACK IT Challenge IVIVE and complimentary awards by the MRC (PhD Case award with AstraZeneca) and BBSRC (PhD case award with Syngenta), the mathematical biology research group in the Data Science Research centre led the development and assessment of a number of 3D microtissue models for liver toxicity testing. In vitro model development was underpinned by metabolic and physiological read-outs, also informed by mathematical, systems and extrapolation modelling at cellular scale and tissue scale.

The liver is a multifunctional organ that is zonated in terms of the existence of spatial gradients in solutes (e.g. oxygen) along fundamental sub-units of the liver microarchitecture (the liver sinusoid). This leads to the spatial separation of an immense spectrum of different metabolic pathways, which is fundamental for proper functioning of this organ. However, modelling a zonated liver lobule in standard culture conditions has not been possible. The aim of our work has been to use a combined mathematical and experimental approach to design three-dimensional cultures that support this critical *in vivo* feature.

Our work focussed on the development and optimisation of three novel microtissue systems, namely: (1) a hollow fibre bioreactor (developed by project partners at the University of Bath, Dr Marianne Ellis); (2) the Kirkstall flow system (with partners at the University of Liverpool, Dr Parveen Sharma; and (3) hepatic spheroids (with partners at the University of Sheffield, Dr Helen Colley and Prof Craig Murdoch). In each of these three systems the aim was to identify the optimal set-up and culture conditions that best mimic the spatial gradients that exist within the *in vivo* sinusoid microenvironment. This work was also underpinned by collaboration with industry sponsors (AstraZeneca, Unilever and Syngenta) who provided in-kind support (in the form of advice for project management, modelling, risk assessment, industry insight and toxicology, access to relevant human, animal and in vitro data or access to specialized technologies) as well as additional monetary support.

In each case the mathematics was led by Dr. Steve Webb in collaboration with researchers from the University of Loughborough (Dr John Ward) and University College London (Dr Rebecca Shipley). We took a multiscale approach, starting at the intra-cellular/cell scale to describe metabolism and clearance of chosen test compounds, then to the scale of the individual bioreactor (incorporating the appropriate bioreactor geometry and appropriate fluid flow dynamics), then extrapolations to real liver and whole body scales were also made to allow IVIVE predictions and comparisons. The role of this mathematical analysis was three-fold:

1) to aid the development of the in vitro system (i.e. by using the mathematical models to predict optimal operating conditions to best recapitulate the zonated liver physiology) and predict system sensitivities and robustness;
2) to help analyse data outputs from the in vitro system and bench mark these outputs against traditional 2D in vitro liver systems as well as other novel 3D systems;
3) to help bench mark the in vitro system outputs against *in vivo* data and to predict confidence levels and uncertainties associated with resulting human predictions.

Results from this mathematical analysis then fed into the subsequent design of the in vitro system set-ups.

Outputs from this research have been published in **[1]-[6]**.

3. References to the research

All publications have been through a rigours peer-review process prior to publication.

[1] Gaskell, H, Sharma, P, Colley, HE, Murdoch, C, Williams, DP, **Webb, SD** (2016) Characterization of a functional C3A liver spheroid model. Toxicol. Res., 5: 1053-1065 **[DOI: 10.1039/C6TX00101G]**

[2] Tomlinson, L, Hyndman, L, Firman, JW, Bently, R, Kyffin, JA, **Webb, SD**, McGinty, S, Sharma, P (2019) In vitro liver zonation of primary rat hepatocytes. Front. Bioeng. Biotechnol., 7:17 **[DOI: 10.3389/fbioe.2019.00017]**

[3] Kyffin, JA, Sharma, P, Leedale, J, Colley, H, Murdoch, C, Harding, AL, Mistry, P, **Webb, SD** (2019) Characterisation of a functional rat hepatocyte spheroid model. Toxicology in Vitro, 55:160–172 **[DOI: 10.1016/j.tiv.2018.12.014]**

[4] Kyffin, JA, Sharma, P, Leedale, J, Colley, HE, Murdoch, C, Mistry, P, **Webb, SD** (2018) Impact of cell types and culture methods on the functionality of in vitro liver systems – A review of cell systems for hepatotoxicity assessment. Toxicology in Vitro, 48:262–275 **[DOI: 10.1016/j.tiv.2018.01.023]**

[5] Sorrell, I, Shipley, RJ, Regan, S, Gardner, I, Storm, MP, Ellis, M, Ward, J, Williams, D, Mistry, P, Salazar, JD, Scott, A, **Webb, SD** (2019) Mathematical modelling of a liver hollow fibre bioreactor. Journal of Theoretical Biology, 475:25-33 **[DOI: 10.1016/j.jtbi.2019.05.008]**

[6] Leedale, J, Colley, HE, Gaskell, H, Williams, DP, Bearon, RN, Chadwick, AE, Murdoch, C, **Webb, SD** (2019) In silico-guided optimisation of oxygen gradients in hepatic spheroids. Computational Toxicology, 12 (November 2019) **[DOI: 10.1016/j.comtox.2019.100093]**

4. Details of the impact

The C3A hepatic cell line 3D spheroid model that was biologically characterised and published in **[1]** gave AstraZeneca confidence in the cell line in spheroid form to further evaluate and develop this model for human-relevant hepatic safety screening. Compared to the conventional 2D – monolayer model, the 3D spheroid model possesses a more *in vivo*-like structure, direct 3D cell-cell contacts, improved zonation, structural and functional polarisation.

Metabolic and physiological read-outs e.g. parameters such as morphological analysis, zonation analysis and protein analysis, from each of the optimised systems were compared. The comparison indicated that each of the 3 systems has its strengths and weaknesses but in terms of the cost-benefit ratio, the results from the hepatic spheroid system (experimental results published in **[1]** with the accompanying mathematics in **[6]**) was attractive enough for AstraZeneca to take this system forward as a first-tier testing tool for hepatotoxicity potential. Feedback from our AstraZeneca industry partners was that our research (described above) gave them confidence in the spheroid form to further evaluate and develop this model for human-relevant hepatic safety screening.

The decision for AstraZeneca to implement this model was taken by Dr Dominic Williams, Associate Director for Preclinical Hepatic Safety, who also made a portion of the AstraZeneca research advisory team.

The model has since been integrated by AstraZeneca into an automated hepatic safety screen for use in early discovery safety. When compared to an equivalent primary hepatocyte assay, the model performs with similar sensitivity and specificity in the detection of potential hepatotoxins. Moreover, it has a higher throughput (56 compounds every 2 weeks), faster turnaround time (2 weeks), lower cost (50x cost reduction) and lacks the donor variation, so improving the consistency of the results.

The assay has been in use globally by AstraZeneca since 2016 to screen approximately 5000 compounds and is impactful in choosing the safety molecules to progress with regards to hepatic safety at the pharmaceutical discovery phase **[A]**. Ultimately, the 3D Spheroid model developed possessed superior liver-specific functionality i.e. ability to synthesise and secrete urea and albumen, functional canalicular transporters and CYP2E1 expression, and increased sensitivity to toxicological response compared to the standard 2D liver models **[1]**.

Consequently, the adoption of this 3D spheroid model has improved AstraZeneca's drug discovery and drug safety selection pipeline, reducing the risk of drug induced liver injury.

5. Sources to corroborate the impact

[A] Letter of support from Dominic Williams (Associate Director for Preclinical Hepatic Safety, AstraZeneca).