



Funded by the Seventh Framework
Programme of the European Union



Project full title:

Hepatic and Cardiac Toxicity Systems modelling

Project acronym:

HeCaToS

Collaborative project

HEALTH.2013.1.3.-1:

Modelling toxic response in case studies for predictive human safety assessment

FP7-HEALTH-2013-INNOVATION-1-602156-HeCaToS

Deliverable Report D8.4:

**Data set describing the impact of drug treatment on the cytokine release of
both 3D models**

Edited by

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Work package 8

Due date of deliverable: M48

Actual submission date: October 2017

Start date of project: October, 2013

Duration: 60 months

Maastricht University (UM)

Project co-funded by the European Commission within the 7th Framework Programme (2013-2018)		
Dissemination Level		
PU	Public	X
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Contributions to deliverable - Internal review procedure

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PUBLISHABLE SUMMARY

Report describing work towards the generation of data on the cytokine release from relevant 3D models treated with drugs on the project drug list.

OBJECTIVES

Using the methods developed through Deliverable Report D8.1, to measure the cytokine release from spheroids provided by WP05.

INTRODUCTION

The objective of the deliverable was to assess cytokine release from spheroids treated with drugs on the project drug list. Spheroids were to be supplied by WP05. This was to be assessed using either a ELISA-based cytokine or IL-3 release assay as developed through D8.1. These kits use a capture antibody and a biotinilated secondary antibody, which is detected in turn by a Streptavidin-HRP conjugate. The data presented in Figure 1 demonstrates that these kits, originally developed for 2D cell cultures, are also applicable to scaffold-free 3-dimensional microtissues. Measurements performed as outlined in SOP 6 and 7. These measurements are applicable to the *in vitro* liver model as it is a kupffer cell co-culture thereby representing valid inflammation model. However, as the main contributor to inflammatory response is immune-derived, measuring release from the cardiac model, while it may be possible technically in very narrow circumstances, was deemed not to significantly advance understanding of the *in vivo* situation as this, where applicable *in vivo*, is only a very minor contributor. The cardiac microtissues are therefore not considered an appropriate inflammation model for these analyses.

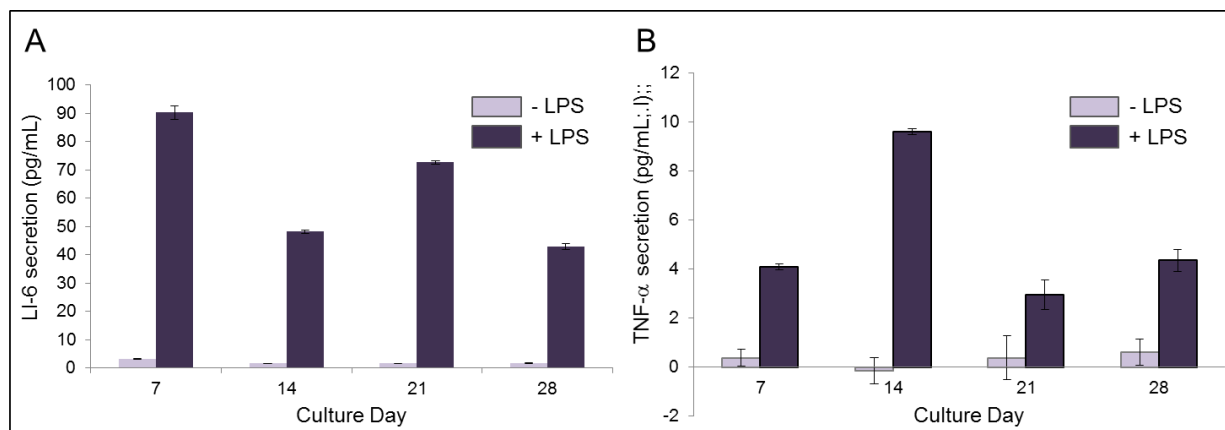


Figure 1: 3D human liver microtissues cultured for increasing periods and incubated with LPS for 24 hours prior to measurement for IL-3 (**A, SOP 6**) and TNF- α secretion (**B, SOP 7**). Medium from 6 wells are pooled for testing.

RESULTS

During the early stages of the project, the consortium chose to adopt an innovative repeat dose 14 day dosing scheme impacting likely spheroid consumption per drug. Also, as analysis methods were

optimised across WP07 and 8, the number of spheroids required per endpoint was more accurately determined. The combination of these activities revealed a greater project demand for spheroids.

To rationalise efforts within the consortium, partner InSphero, originally tasked with generating these data, focused resources on WP05 activities (spheroid provision) to:

- (i) reduce spheroid consumption and
- (ii) to maintain supply of spheroids to the consortium at the levels required to facilitate a complex repeat-dose 14 day exposure analysis across all of the endpoint deployed.

Mitigating this change was the fact that it was felt by the consortium that information on inflammatory response could be gleaned from proteomic analysis. For these reasons, this endpoint was not pursued as an ELISA analysis.

DIFFICULTIES

As outlined above, a combination of the innovative dosing scheme adopted and the number of spheroids required per measurement parameter (WP07 & 8) resulted in an increase project demand for spheroids over budgeted amount. InSphero resources were therefore diverted away from the activity towards spheroid provision for all other measurements. Mitigating this change was the fact that it was felt by the consortium that information on inflammatory response could be gleaned from proteomic analysis. For these reasons, this endpoint was not pursued as an ELISA analysis.