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Enhancements in the Preclinical Investigation of Drug-Induced Liver Injury

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Abstract

Drug induced liver injury is a major problem for the pharmaceutical industry and health services. Yet 30% to 40% of human hepatotoxins go undetected during *in vitro* studies. Hence, more predictive *in vitro* liver models are a critical requirement for preclinical screening of compounds demonstrating hepatotoxic liability. Huge advancements have taken place over the last decade innovating cell culture techniques in order to improve function of predictability of *in vitro* toxicity models. This review summarizes some of the key advancements in models used for the preclinical investigation of drug-induced liver injury.

Introduction

Exciting advancements have been taking place in preclinical screening. Researchers are investigating the potential of novel, complex *in vitro* systems, fuelled by the ever increasing failures during drug trials. Simple 2D monoculture cell models were becoming outdated, unable to detect up to 40% of hepatotoxic compounds and costing companies precious time and money and with improvements in tissue culture and engineering, novel techniques were becoming more easily available [1]. Figure 1 depicts some of the most common systems in which Drug-Induced Liver Injury (DILI) is investigated.

Numerous liver models are available for the investigation of DILI, these models range from the closest resemblance to humans with highest complexity to the most ethically accepted and easy to manipulate systems. With advancements in 3D cell culture, spheroid models have now been optimized and validated for use in drug safety screening, with numerous advantages over older *in vitro* models.

Clinical research into human DILI is essential; furthermore, animal studies are legally required to be performed on two separate species before a chemical can be tested in patients [2]. However, it is clear that *in vitro* models are absolutely crucial in order to detect human hepatotoxicity before entering clinical trials. These liver models tend to be human-relevant, inexpensive and with little limitation as to what experiments can be carried out. However, in the past, *in vitro* liver models have lacked complexity, resulting in systems which do not recapitulate human liver structure or function and consequently poorly predict hepatotoxicity [3].

Researchers have attempted numerous techniques in order to try and improve liver models, for instance investigating multiple different cell types such as primary hepatocytes, modified cell lines, stem cells, co-cultures as well as culturing with ECM or scaffolds [4]. For example, sandwich culture of hepatocytes can prolong their function and produce secondary structures. However, these models still have disadvantages, they do not represent a human liver, and have problems with variability and impracticalities performing the techniques [1,4]. One of the most significant changes to *in vitro* drug testing was undoubtedly the emergence of 3D models. Spheroids, bioreactors and scaffolds have been used for many years in embryonic research and cancer therapeutics and more recently adapted in other areas of drug research [5]. Numerous research groups are now investigating the use of 3D liver models for drug testing, aiming to create organ-like structures in order to detect toxicity of novel drug compounds. 3D liver cell culture has proven on numerous occasions to display more *in vivo*-like structural and functional components when compared to monolayer cultures, correlating with an increased sensitivity to toxicants [4,6-8].

Spheroids appear to have become the one of most popular novel *in vitro* liver models, with multiple companies, such as InSphero and Organovo, now offering pre-formed spheroids to be shipped directly for drug screening purposes. With the advantages of being easier to create and

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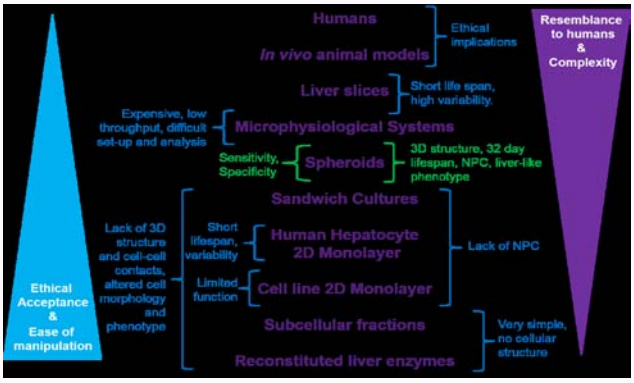

Figure 1: Advancements in liver models.

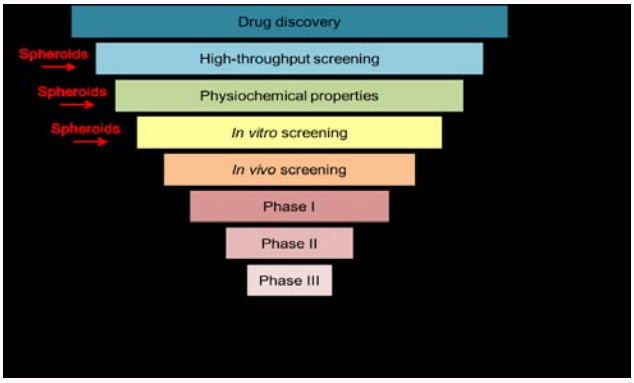

Figure 2: The drug development process.

manipulate than more complex novel cell culture techniques, spheroids are an attractive model to bridge the gap between the very simple and overly complex liver models. Furthermore, spheroids created from liver cell lines have the benefits of being more widely available and with less ethical implications than using liver slices or primary cells. These improvements are mirrored in the ability of liver spheroids to detect human hepatotoxins [6-12]. Hence, liver spheroids have immense potential for investigating DILI.

Potential of C3A liver spheroid model for the investigation of DILI

How does the C3A spheroid model compare to other *in vitro* liver models? With increasingly complex cell culture models emerging, how do C3A spheroids fit into the drug screening process? Figure 2 depicts the important stages in the development of a novel drug compound. *In vitro* models are used in the drug development process to investigate the efficacy of a lead compound, as well as determine its kinetics and safety in humans. It is impossible to choose one perfect model for drug screening; one can however choose an appropriate model, or combination, in order to answer a specific research question.

The development of a novel therapeutic compound firstly involves the discovery of a novel drug compound. This involved the identification of a lead compound with efficacy for the target of interest using high-throughput screening and analyzing physicochemical properties. The next stage, lead optimization, consists of *in vitro* and *in vivo* testing of these compounds to ensure safety and efficacy of the compound. Finally, clinical trials are performed before the drug can be marketed for clinical use. Spheroids could potentially be utilized at multiple stages of the drug development.

For preclinical drug screening, an initial simple but effective screen is required to identify lead compounds. This drug screen must ideally be fast, high-throughput, inexpensive, reproducible, readily available and easy to analyze. The C3A spheroid model can be created in 72 hours, in 96-well plate format, from an inexpensive and reproducible cell line, with easy end-point analysis automated production and dosing of C3A spheroids is possible, further increasing the ease of use of this model [9]. The biology of the C3A spheroids has been validated, with key parameters elucidated including 32 day lifespan, cell viability, proliferation rate, cell number, diameter, 3D cell morphology and secondary structures [9]. Furthermore, the analysis of liver-specific functions of C3A spheroids revealed an increased likeness to *in vivo* functionality. In addition to this, C3A spheroids display >90% sensitivity and specificity to hepatotoxins (unpublished data) [9]. This evidence suggests the potential of a C3A spheroid model to detect hepatotoxins and improve upon the sensitivity of 2D culture models. Hence, C3A spheroids are a promising, novel, human-relevant *in vitro* model for high-throughput drug screening.

In addition, spheroids could be used further down the drug safety process. The C3A spheroid model can be cultured for at least 32 days, allowing for longer and repeated drug exposures, with the potential of analysing sub-acute or delayed drug effects. For a more in-depth analysis the spheroid model could be utilized to investigate the mechanism by which compounds are causing toxicity by analyzing novel biomarkers of DILI, mitochondrial function, transporter inhibition or the effect of the compound on NPC. There is even the potential to use more complex spheroids to model liver diseases and examine the efficacy of therapeutic compounds.

Future potential for *in vitro* liver models

In the future, *in vitro* liver models are likely to become more advanced. Multi-compartmental micro fluidics devices are now freely available, allowing the flow of media from one cell model to another, replicating the interaction of multiple different cell types, tissues or organs of the human body. This could involve compartments containing different NPC, allowing the circulation of soluble factors between the different cell types, or indeed the interaction of multiple organ systems with the liver compartment. Liver spheroids could be incorporated into a multi-compartmental model in order to recapitulate a human liver more precisely than 2D cultured cells. Studies have attempted to combine 3D spheroids with flow and the combination of these two techniques could further improve liver-specific functions and result in increased sensitivity to hepatotoxins, once practical difficulties are overcome [8,13,14]. Novel 3D models have the ability to recreate complex structures incorporating multiple cell types and ECM due to the enhancements in chemical engineering and 3D printing. The production of an entire *in vitro* liver sinusoid structure is now possible, with a capillary compartment supplying a flow of oxygen and nutrients, a 3D mass of hepatocytes and NPC in the sinusoid themselves, secondary structures and a bile compartment to remove waste products produced by the hepatocytes [1,12,15]. Furthermore, in order to overcome some of the practical difficulties of using specialist *in vitro* models, these systems are likely to become more automated, with continuous monitoring of important parameters as well as automated sample collection or imaging in order to become more user friendly and produce higher volumes of data [16]. The analysis of these systems is likely to also become more specialized, with technologies such as high-content

screening becoming available and entirely automated analysis of end-points, the quantity of data obtained from these drug screens is ever increasing and these analyses have the potential to have a higher predictivity than *in vivo* models [17-19].

The possibility of replacing animal models with more human-relevant 3D models has been discussed, in order to improve on the 50% of human hepatotoxins detected in animal studies [20]. *In vivo* experimentation will always receive criticism due to the unethical nature of causing harm to animals. Furthermore the vast genetic and environmental differences beckon the question as to whether these animal models are relevant to human toxicity [2]. Complex human-relevant *in vitro* liver models have immense potential to be used to analyze drug efficacy, ADME (Adsorption, Drug Distribution, Metabolism, Excretion) and side effects, all of which could help determine a safe and efficacious dose for clinical trials [16,17,19]. The advantage of micro fluidics offers more realistic thermodynamic properties of the model, allowing estimations of compounds exposures [10,13,16,21-23]. Furthermore, complex liver models could be used to investigate drug toxicity more intricately, with the possibility to model interactions between multiple organ systems and investigate the mechanisms of drug pharmacology and toxicity in detail [16,22]. Therefore, although abolishing *in vivo* studies completely is unlikely, many animal-based assays could be replaced with an alternative *in vitro* test system. In the future these specialized liver models may have a vital role throughout the drug development process.

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