"Future of *In vitro* Cell Biology".

2D | | 3D
Cell monolayer | VS | Spheroids | Organoid | OoC
Traditional method of cell culturing

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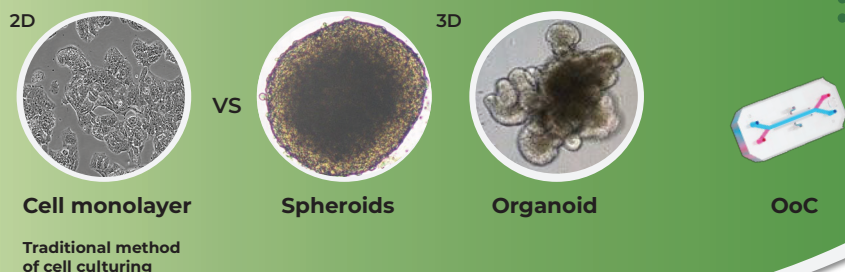
APPLICATION OF 3D HEPATIC SPHEROID MODEL IN ASSESSMENT OF LONG-TERM HEPATOTOXICITY

Safety evaluation has been undertaken using laboratory animals for a while. Existing animal models as well as two-dimensional cell monolayer *in vitro* systems used for screening of xenobiotics are not able to completely mimic the complex human system. Cells in a monolayer are mostly deprived of cell–cell and cell–extracellular matrix interactions[1]. Employing such models for the screening of xenobiotics is prone to give misleading results, often leading to the withdrawal of drugs during the clinical trial phase.

Features lacking in 2D cell monolayers like cell-extracellular matrix interactions, tissue-specific architecture[2], etc. are present in three-dimensional (3D) cell culture models like spheroids, and organoids. The cytology and morphology of the spheroids better represent the *in vivo* tissue organization and microenvironment than the traditional 2D monolayer cell cultures. Unlike the 2D models that can be cultured for a definite period, cells in a 3D model can survive for a long time in culture. Owing to its extended survival period, 3D models may serve as a useful tool in studying the long-term effects of xenobiotics. 3D spheroid culture can help significantly in improving cell viability[3], in turn allowing long-term *in vitro* toxicity screening with repeated dosing.

Drug-induced liver injury (DILI) / hepatotoxicity is one of the leading causes of withdrawal of drugs in the early drug discovery process. Application of the 3D spheroid models in studying the underlying condition can help in better understanding the *in vivo* outcome, upon human exposure to drugs.

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A cell biologist with 8 years of experience in *in-vitro* assay development and GLP validations.

She is actively involved in developing various assays in the areas of sensitisation, endocrine disruption, generation of 3D models and is a key member in technology transfer.
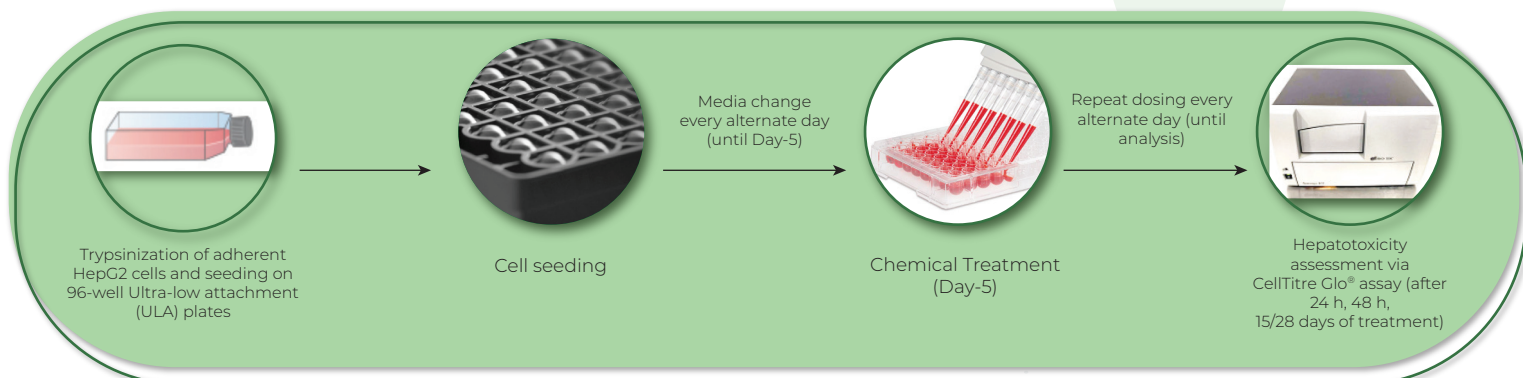
Our present study is aimed at generating hepatic spheroids by employing cells of hepatic origin, viz, HepG2 cell line, and understanding the toxicity profile of drugs known to cause severe liver injury / hepatotoxicity (i.e., Acetaminophen, Diclofenac, and Amiodarone) to no effect (i.e., Streptomycin), as presented in FIGURE 1. Each chosen drug belonged to a different category, viz., Streptomycin (an anti-bacterial drug), Acetaminophen (non-opioid analgesic and antipyretic agent), Diclofenac (Non-Steroidal Anti-Inflammatory drug), and Amiodarone (an antiarrhythmic drug).

Trypsinization of adherent HepG2 cells and seeding on 96-well Ultra-low attachment (ULA) plates → Cell seeding → *Media change every alternate day (until Day-5)* → Chemical Treatment (Day-5) → *Repeat dosing every alternate day (until analysis)* → Hepatotoxicity assessment via CellTitre Glo® assay (after 24 h, 48 h, 15/28 days of treatment)

FIGURE 1: Schematic representation of Hepatotoxicity assessment via CellTitre Glo® assay.

HepG2 cells were seeded onto 96-well ULA plates for the formation of spheroids, with media changes performed every alternate day (until Day-5). On day-5, spheroids were treated with test chemicals. Repeat dosing with test chemicals was performed every alternate day until the day of hepatotoxicity assessment. To assess hepatotoxicity induced by the test chemical, CellTitre Glo® assay was performed and the luminescence was recorded using a luminometer.
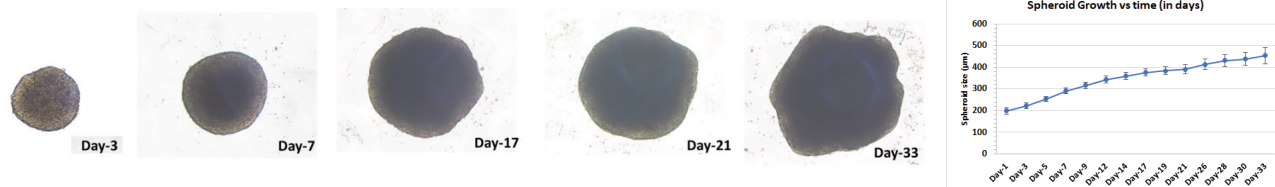
FIGURE 2: Growth of spheroid with respect to duration of culture (time in days).

The average size of spheroid increased from ~ 200 μm (Day-3) to ~450 μm (Day-33) over 4.7-weeks culture duration. A 2-fold increase in the spheroid size was observed over the 33 days of culture duration (FIGURE 2).

We tested the hepatotoxic effect of Streptomycin (a known non-DILI / non-hepatotoxic drug) and Acetaminophen, Diclofenac, and Amiodarone (DILI causing/hepatotoxic drug) at different concentrations to spheroids for 24 h, 72 h, 15 days and 28 days duration.

- We observed Streptomycin showing no signs of toxicity even after 28 days of repeat exposure with an IC_{50} value >5000 μM for all time points.
- Acetaminophen, Diclofenac, and Amiodarone showed no signs of toxicity (with cell viability >50%) upon 24 h of exposure to spheroids, while repeat exposure upto 72 h, 15, and 28 days clearly identified these compounds as hepatotoxic agents (FIGURE 3).
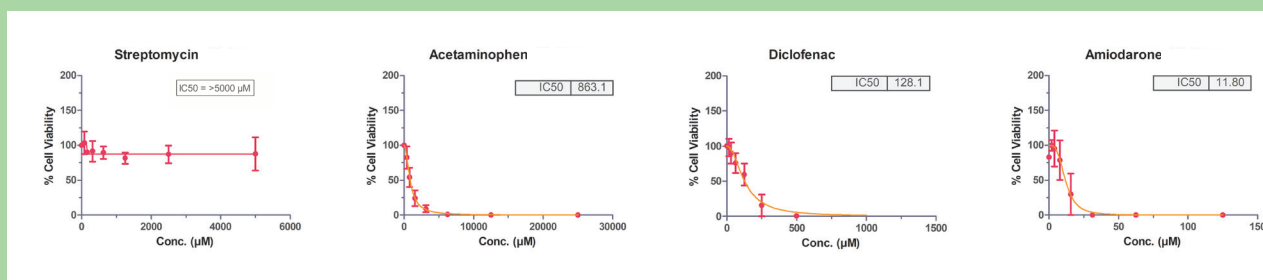

FIGURE 3: Dose-response curve of spheroids upon 28-days exposure with the test items, i.e., Streptomycin, Acetaminophen, Diclofenac, and Amiodarone, followed by viability assessment via CellTitre Glo® assay.

BENEFITS / APPLICATION OF THE 3D SPHEROID MODEL

The developed model is a valuable tool in predicting long-term hepatotoxicity, a feature that cannot be studied using 2D cell culture due to their shorter culture duration and this in turn limits its use in any long-term study. On the contrary, cells in a 3D model show reduced proliferation with the capacity to proliferate getting ceased over time. Therefore, the lower proliferation rate of 3D models can be effectively exploited for studying the effects of long-term exposure to various xenobiotics, which is not feasible in a 2D model.

We at Jai Research Foundation are committed to eliminating the redundant use of animals in compliance with the 3Rs. Thus, we are happy to offer this *in vitro* 3D model that uses cell lines to reduce animal usage in understanding underlying liver conditions.

CONCLUSION

3D models may be useful during pre-clinical assessment for studying underlying liver conditions and may also help in predicting the *in vivo* outcome upon exposure to drugs.

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