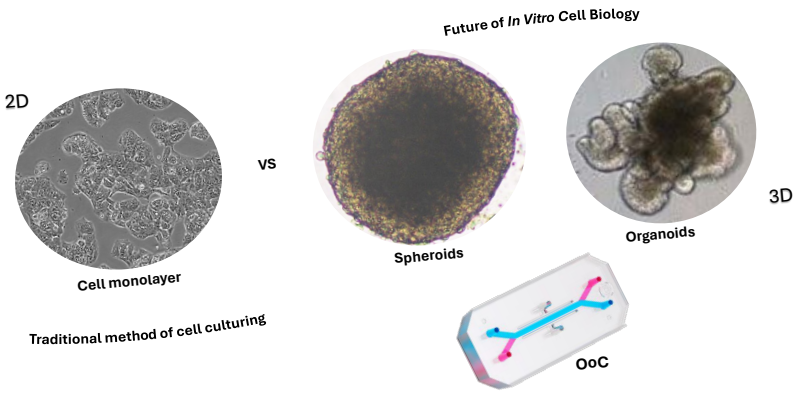

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A cell biologist with 8 years of experience in in-vitro assay development and GLP validations. She is actively involved in developing various assays in the areas of sensitisation, endocrine disruption, generation of 3D models and is a key member in technology transfer.

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Evaluation of Non-Alcoholic Steatohepatitis (NASH) and CYP induction using 3D hepatic spheroid model

Non-Alcoholic Steatohepatitis (NASH) is the most common liver disorder characterized by abnormal hepatic accumulation of fat leading to inflammation[1]. Despite its high prevalence, the biology of progression of the disease is unclear and due to which there are no FDA approved drugs available to treat this condition.

CYPs are enzymes that play an important role in drug metabolism. Clinical drug-drug interactions (DDI) are mostly prevalent due to induction of CYP enzymes. Therefore, CYP induction is one of the parameters that the drug candidates are screened for early in the drug discovery process.

Various cellular models like 2D monolayers, primary human hepatocytes (PHH), etc. are employed during pre-clinical testing for assessing NASH and CYP induction potential of a drug. However, as 2D models are not able to mimic the complexity of the natural tissue microenvironment and PHH being uneconomical, limits its usage as a cell culture model for screening of xenobiotics. Thus, there is a critical need to develop advanced *in vitro* cellular systems like 3D models which may help in studying these disease mechanisms and might also be of help in identifying drug responses.

Our present study is aimed at generating 3D liver spheroids using HepG2 cells and understanding the applicability of the model in studying liver anomalies, i.e., NASH and CYP induction.

Methodology

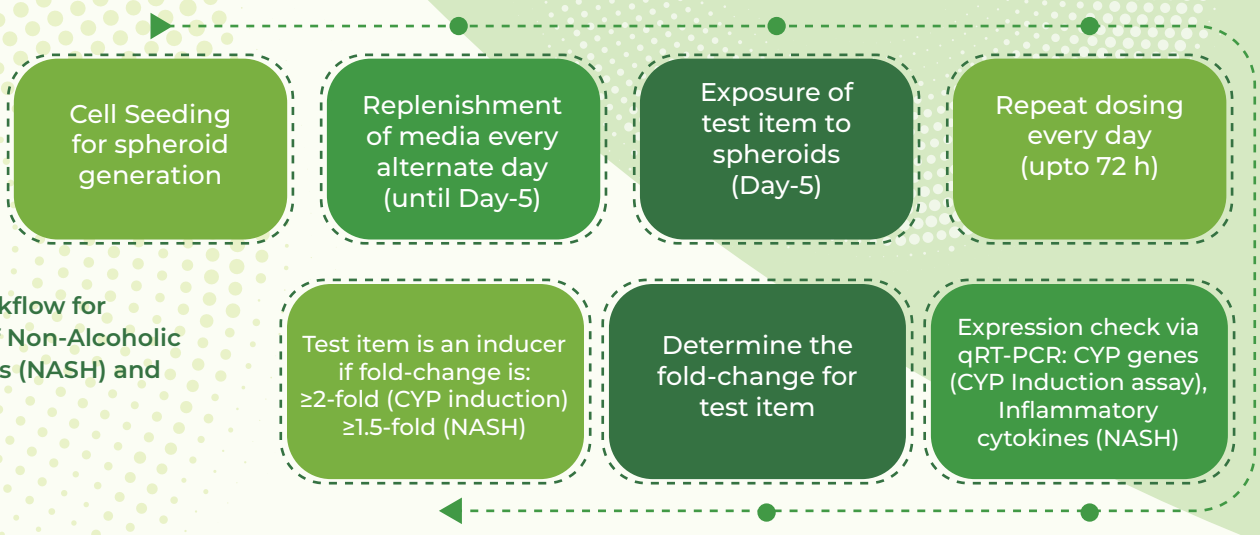
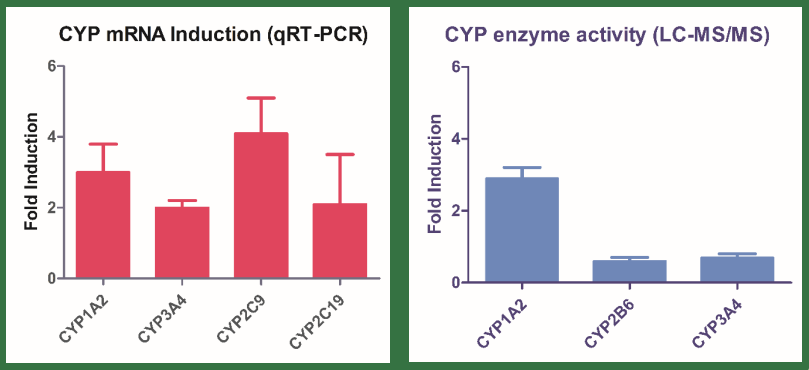


FIGURE 1: Workflow for assessment of Non-Alcoholic Steatohepatitis (NASH) and CYP induction

Results

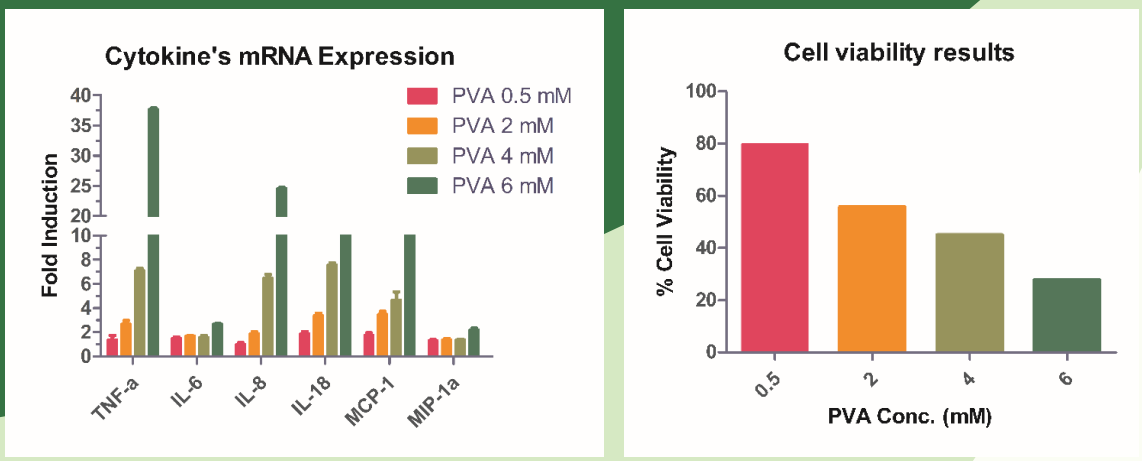
With regards to the CYP induction assay, mRNA induction above 2-fold was observed in case of CYP1A2, 3A4, 2C9, and 2C19 as compared to the vehicle control, 0.1% DMSO (Figure 2a). At the enzyme activity level, induction >2-fold was observed in case of CYP1A2 (Figure 2b).

With regards to Non-Alcoholic Steatohepatitis (NASH), upon treatment with 2-Propylvaleric acid (PVA), an increase in the expression of TNF-α, IL-6, IL-8, IL-18, MCP-1, MIP-1α, above 1.5-fold was observed at the transcriptional level (Figure 3a). Cell viability was in the range of 27.8% to 79.8% (Figure 3b). Based on our findings, PVA is concluded as a potential inducer of NASH.



(a) (b)

FIGURE 2: CYP Induction in response to positive controls. CYP mRNA induction via qRT-PCR method (a) catalytic/enzyme activity measurement using LC-MS/MS (b).



(a) (b)

FIGURE 3: NASH assay - mRNA induction in response to 2-Propylvaleric acid. Cytokine expression assessment via qRT-PCR (a) cell viability assessed via CellTitre Glo® assay (b).

Conclusion

Preliminary findings emphasize the model's feasibility during preclinical screening in assessing inducers of CYPs and NASH. Further studies may help in exploring its applicability in studying other underlying liver conditions and may also help in predicting the *in vivo* outcome upon exposure to drugs.

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