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**Title:** Bioengineered humanized livers as better three-dimensional drug testing model system

**Column:** Basic Study

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Dear Editor,

We are herewith submitting and confirming the required revisions in our manuscript for your kind perusal.

**Step 1** **36450-ORCID numbers**

**All the authors have confirmed their ORCID numbers as follows:**

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**Step 3 36450-Scientific research process**

**1. What dis this study explored?**

The present study was undertaken to develop more appropriate humanized ex-vivo model system to overcome the limitations of available strategies.Bioengineering humanized livers developed herein explored a way towards the development of suitable humanized preclinical model systems for pharmacological testing. This approach may reduce the cost and time duration of preclinical drug testing and further overcomes on the anatomical and physiological variations in xenogeneic systems. It provides enhanced dose response relationship by using drug concentrations relative to human exposure. Ease of ex vivo access of cellular and molecular responses in humanized liver model system during pharmacological screening also offers high-throughput studies to determine the cellular response networks and toxicity pathways.

**2. How did the authors perform all experiments?**

Bioengineering humanized livers were developed in this study using human hepatic stem cells repopulation within the acellularized liver scaffolds which mimics with the natural organ anatomy and physiology. Six CYP probes were used to enable efficient identification of drugmetabolism in bioengineered humanized livers. The drug metabolism study in bioengineered livers enabled to evaluate natural drug absorption, distribution, metabolism, excretion and toxicity responses.

**3. How did the authors process all experimental data?**

All the data of present study were expressed as Mean±SEM. Each experiment was performed in triplicate in two separate cohort studies to maintain the reproducibility. During metabolism studies, are of the drug was divided by the area of internal standard to calculate the area ratio. One way and two way ANOVA was performed using Graph Pad Prism (version V) to identify the statistical significance among multiple groups. *p*<0.05 was set as statistical significance for all the variables in different groups.

**4. How did the authors deal with the pre-study hypothesis?**

Human liver play significant role in drug metabolism and toxicological response. Therefore drug-induced liver toxicity has been a major concern for the development of acute liver failure and post-market drug withdrawal due to the absence of suitable humanized preclinical model system. Animal models have been the gold standard platform to identify the toxicological effects of pharmacological drugs/molecules. However, species difference always does not allow predictive outcome similar to human system. Hence, several in vitro models of human livers have been developed to complement the animal model system. The most widely used in vitro models include human liver specific cell lines such as HepG2, Hep 3B and SNU-398. However, these cell lines lack expression of several molecular crucial for drug targeting. Due to the enormous potential of humanized tissues/organs in pharmacological studies, several investigations have been focused to generate biomimetic humanized organs which is an urgent need to replace the conventional 2D/or 3D ex vivo systems and animal models to reduce the investigatory and economic burdens towards the preclinical evaluation of drugs. Discovery of stem cells has given a potential hope to regenerate the diseased organs or tissues in human body. Since then, various strategies have been tried to evolve humanized organs and/or tissue using stem cell technology for in vitro discoveries and in vivo transplantation studies.Despite these significant advances, several crucial issues related to drug absorption, distribution, metabolism, excretion and toxicity (ADMET) indicates lack of sufficient predictability in drug evaluation models. To avoid such higher failure rate in late-stages of drug testing processes, more appropriate humanized platform is highly desirable to generate better preclinical outcome.

The bioengineered humanized model developed in this study provides natural system for above described assumptions which could be more practical approach to replace the earlier developed models including animals. In addition to the natural architecture, presence of human primary hepatocytes provides activities ofhuman liver metabolic enzymes to identify the real pharmacokinetics of drugs. Food and Drug Administration (FDA) guidance requires more than 25% clearance from the CYP mediated liver metabolism prior to conduct human trial on a particular drug. As CYP is the most common group of enzymes found in liver for the clearance of drugs, it has been proposed better pathway to study the drug metabolism.The metabolic study of six CYP substrates in present investigation using bioengineered liver system could provide better platform for future drug metabolism studies as a replacement of animal models as unique preclinical model system. This unique system offers several advantages over the conventional models of drug metabolism studies:

**5. What are the novel findings of this study?**

Humanized liver model system could be ideal choice for drug metabolism studies using tissue specific 3D-architecture, proper cell to cell and cell to ECM interactions which make them one of the best model systems to predict the drug responses more likely to human system. The 3D-architecture of this model provides in vivo like context and also eliminates the species differences. This system allows biomimetic humanized preclinical outcomes by allowing natural drug delivery and distribution. Bioengineered humanized livers could be more suitable option for determining drug safety and efficacy in human mimetic preclinical model system. This unique biomimetic platform can produce better outcome during disease modelling and ADMET studies.

**Step 4 36450-Academic Rules and Norms of This Article**

**(1) 36450-Institutional review board statement**

The present study was approved by the Institutional Review Board and Ethics Committee of Deccan College of Medical Sciences, Hyderabad.(Attached)

**(2) 36450-Institutional animal care and use committee statement**

The utilization of animals for conducting this study was approved by the Institutional Animal Ethics Committee of Deccan College of Medical Sciences, Hyderabad.(Attached)

**(3) 36450-Animal care and use statement**

All experiments of this study were conducted according to the ethical and regulatory guidelines of Indian Council of Medical Research (ICMR), India.

**(4) 36450-Biostatistics statement**

All the statistical analysis in this study were performed using standard methods and softwares. Statistical significance were generated using either column stats, one way or two way anova using graph pad prism software (version 5).

**(5) 36450-Conflict-of-interest statement**

All the authors have declared that they do not have any conflict of interest towards the publication of this manuscript.

**(6) 36450-Language certificate**

This is to inform you that all the authors are native of India. Wherein we have enough qualification and expertise on scientific writing of research data in English. Till date we have published more than 200 articles in English. So we don’t think English language certificate is a mandatory policy for Indian authors for paper publication in English. In addition, our paper has been edited by one of the expert in manuscript scientific writing.

**(7) 36450-Approved grant application form(s) or funding agency copy of any approval document(s)**

There was no external funding was involved to conduct this study.