The Use of iPSC-Derived Liver Organoids as an Exclusive Toxicity Testing Tool

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Abstract: Drug-induced liver injury (DILI) remains a primary reason for drug withdrawal from the market, often after large amounts of money have been invested and patients put at risk in clinical trials. In dealing with DILI, the current 2D models are not sufficient in predicting DILI, thereby resulting in DILI discovered in clinical trials and postmarket surveillance. In recent decades, organoid technology has gained much attention and interest. The self-organizing and self-renewing features of organoids has led to their application in disease modeling, regenerative and personalized medicine, as well as in toxicity testing. The emergence of organoid technology challenges current *in vitro* and *in vivo* toxicity testing models as it overcomes several drawbacks two dimensional (2D) traditional models face. This review discusses the use of induced human pluripotent stem cells (iPSC) to make liver organoids specifically. Among many sources to make liver organoids, iPSCs are the least invasive and can ensure reproducible productions of liver organoids which better recapitulates the human liver *in vivo*. This paper, in particular, looks at the potential of iPSC-derived liver organoids as an exclusive tool for liver toxicity testing, including liver organoid construction, functionality, hepatic biomarkers measured, commercial availability, and challenges.

Key words: DILI, iPSC, liver organoids, toxicity testing.

1. Introduction

Organoids emerge as a breakthrough technology by their versatility in disease modeling, personalized medicine, regenerative medicine, and drug development [1]. Organoids can incorporate several cell types and are self-renewing and self-organizing and therefore represent an organ of interest *in vitro* [2]. While many recent papers have reviewed iPSC-derived organoids in disease modeling and medicine, very few have shed light on their applications in toxicity testing. The liver is an essential organ for xenobiotic metabolism, which is directly targeted by drugs if toxic [3]. Among the reasons for drug withdrawal in the US and Europe from the 1970s to the 2000s, DILI is the primary one [4]-[6].

The current 2D toxicity testing models often fail to predict DILI which poses a great risk to the public. Unfortunately, about 38-51% of drugs with hepatotoxic potential are not detected in preclinical tests [7]. In light of this, it is crucial to develop new strategies to more accurately predict hepatotoxicity in drugs to reduce DILI occurrence.

The report Toxicity Testing in the 21st Century: A Vision and a Strategy published by the U.S. Academy of

Science in 2007 envisioned the major transition from expensive *in vivo* animal models to *in vitro* human cells and cell lines, combined with high throughput screening for toxicity testing. The report highlights the implementations necessary to realize the vision of predominant *in vitro* testing, one being the *in vitro* tests preferably based on human cells, cell lines, and components that could better resemble the toxicity responses in the human liver. However, over ten years after the report was published, immortalized liver cells and primary hepatocytes are still the major liver cell types used in hepatotoxicity testing [8]. This again urges the need for a better hepatotoxicity testing tool *in vitro* as we are aiming for more accurate predictions with fewer expenses in a shorter period of time.

Available data indicate that iPSC-derived liver organoids are a better toxicity testing tool to distinguish between toxic and therapeutic doses which could lead to acute and chronic DILI given they can, *in vitro* better resemble the human liver *in vivo* [9]-[11]. This paper, in particular, looks at the potential of iPSC-derived liver organoids as an exclusive tool for liver toxicity testing, including liver organoid construction, functionality, hepatic biomarkers measured, commercial availability, and challenges.

Although this paper will focus on acute and chronic DILI, idiosyncratic DILI (IDILI) is also a major cause of DILI that leads to post-market drug withdrawal [5]. Currently available studies demonstrate that liver organoids, as currently prepared, detect at least some IDILI [10]. I will consider the possibility of using modified iPSC-derived liver organoids to predict IDILI, where additional components are added to liver organoids in order to more accurately predict the causes and outcomes of IDILI.

2. Toxicity Testing Models for Hepatotoxicity

DILI can be the result of the intrinsic toxicity of the drug or delayed liver damage without any apparent acute or chronic cause, known as IDILI. The current approach to toxicity testing shown in Table 1 involves an array of toxicological studies both *in vivo* and *in vitro* to evaluate pathological endpoints and clinical signs but are time-consuming and have low human relevance of toxicity responses [12].

Table 1. Summary of Current Toxicity Testing Models [9], [13]-[16]

Current *in vitro/ in vivo* Models	**Benefits**	**Limitations**
Animal models	Active xenobiotics metabolism, Complete ADME profile, Immune system included	Expensive, Time consuming, Low human resemblance
Human liver tissue	Includes all liver cell type, Retains CYP activities up to 96 hours	Invasive, Nonrenewable, Not available for chronic toxicity studies
Primary hepatocytes	Higher human relevance, Higher CYP enzyme level, Closer to *in vivo* liver	Invasive, Rapid decline of CYP levels and morphology
Immortalized liver cells HepG2/HepaRG	Highly proliferative	Short lifespan, Low CYP enzyme level, Insensitive upon hepatotoxic drug induction
iPSC-derived liver organoids	Higher human relevance, Can mimic blood flow, Allow involvement of different cell types	Does not include immune system, Currently not commercially available

Animal models such as mice models are well-known as the “gold standard” for toxicological study as they mimic a complete absorption, digestion, metabolism, and excretion route in humans that cannot otherwise be observed *in vitro* models. However, there have long been discussions about animal to human

extrapolation of toxicological responses not being accurate because mice have a different metabolism profile than humans. Also, toxicity results are not reproducible across species as shown in the US National Toxicology Program analysis of 37 chemicals [17], [18].

Human liver tissue slices, Immortalized liver cells, and primary hepatocytes are *in vitro* liver toxicity testing models that are commonly used. Liver tissue slices are useful models as they include all liver cell types, are well relevant to *in vivo* liver, and retain zonal cytochrome P450 activities for 20-96 hours when cultured [14]. However, obtaining liver slices requires surgery performed on the donor to retrieve and cool the liver (or liver portion) as quickly as possible. Following this, experts' delicate cutting from the liver with confined width and length and following flushes to acquire the sample are also required. Even if the liver slices are reproducible, it is still difficult to find a donor with a "fresh" liver to study in the first place [15], [19]. Immortalized liver cells, such as HepaRG and HepG2 cells, are derived from hepatoma cells. These cells are highly proliferative, but HepG2 cells express much less Cytochrome P (CYP) 450 enzyme activity than primary human hepatocytes (PHHs), thus are not sensitive toward hepatotoxic drugs. HepaRG cells, on the other hand, do express CYP enzymes but still at a lower level than PHHs [16]. PHHs are isolated from liver tissue of patients undergoing tumor resection or from liver donors, hence have limited availability [20]. Even though PHHs have been the gold standard for *in vitro* liver testing, they are invasive to obtain and have a limited functional lifespan of 24-72 hours. Their albumin secretion and CYP enzyme activities decline drastically after 24 hours as PHHs isolated as above gradually lose their structure and cell-to-cell interaction, which are significant for xenobiotics metabolism [14]. Given these hepatotoxicity testing models have drawbacks that do not well resemble *in vivo* human liver, here this paper proposes iPSC-derived liver organoids as the alternative exclusive hepatotoxicity testing tool that can better depict the human liver *in vivo*. Fig. 1a and Fig. 1b show a workflow for *in vitro* and *in vivo* hepatotoxicity testing.

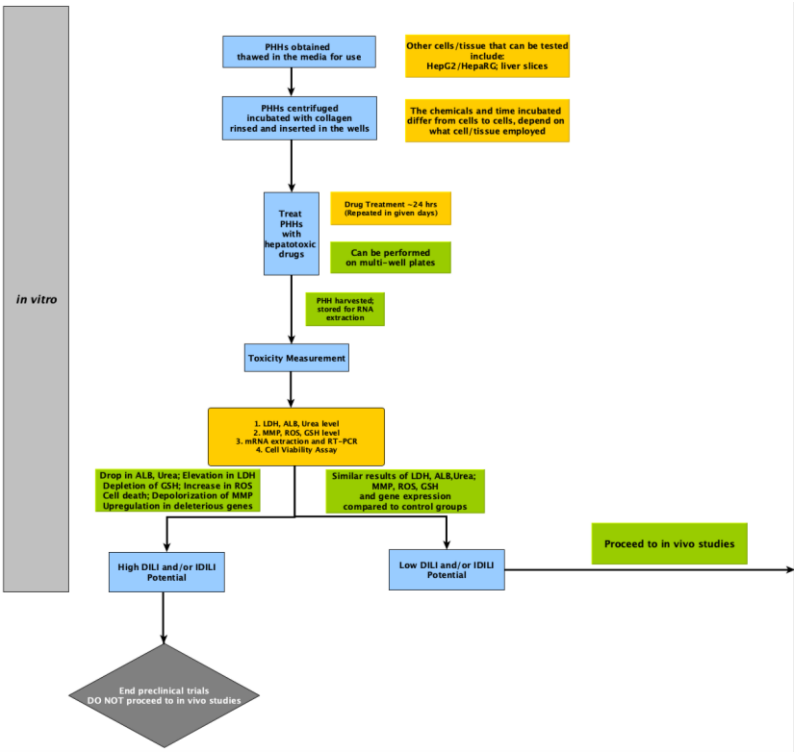

Fig. 1a. Workflow for current *in vitro* hepatotoxicity testing [21]-[25].

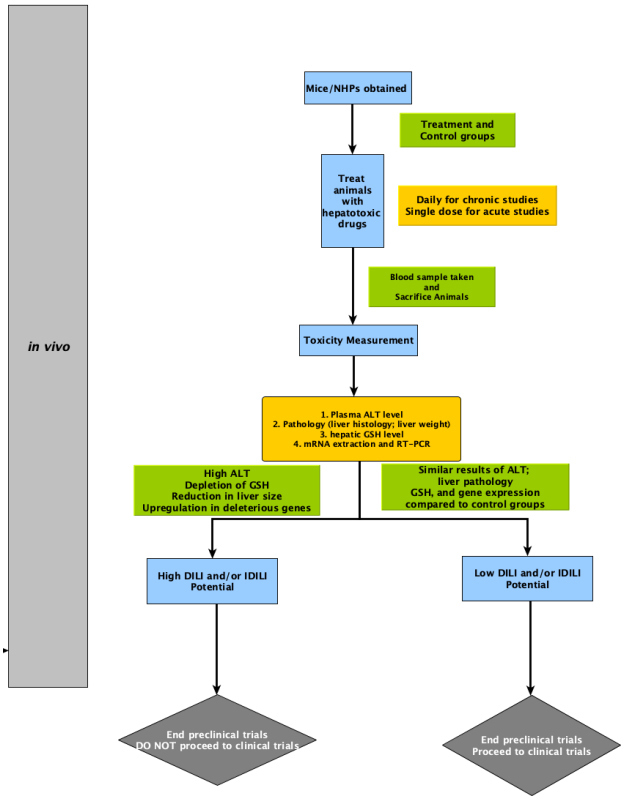

Fig. 1b. Workflow for current *in vivo* hepatotoxicity testing [21]-[25].

3. The 3D Liver Co-culture Liver System

One of the first comprehensive, long-term liver co-culture studies was done by Kostadinova *et al.* in 2013, in which they characterized 3D human co-culture models with four major liver cell types including hepatocytes, kupffer cells, endothelial cells, and stellate cells. This co-culture system was able to preserve liver protein secretions and CYP enzyme levels for three months and was also able to identify hepatotoxic drugs that cause DILI and/or IDILI such as troglitazone, trovafloxacin, and APAP [26]. Their findings show promising results of a 3D liver model in detecting DILI and/or IDILI that are difficult to obtain in conventional toxicity models. However, the Kostadinova group obtained PCs and NPCs from liver tissue donors which requires new liver tissue to construct a new system. This co-culture system is not defined as a liver organoid and is not made from iPSCs, but it opens the possibility of building 3D liver models that well resemble human liver, which allow for accurate DILI and/or IDILI predictions.

4. Building Liver Organoids

Liver organoids are cells that grow in defined three-dimensional (3D) *in vitro* culture which can self-organize and differentiate into specific cell types. These structures can be derived from PHHs, embryonic stem cells (ESCs), adult stem cells (ASCs), and induced pluripotent stem cells (iPSCs). As the ethics of obtaining ESCs are controversial, and PHHs and ASCs are isolated by invasive means, iPSCs are the superior source of organoids currently available [19].

iPSCs are reprogrammed from skin fibroblasts which are collected via superficial skin punch biopsy under patients' local anesthesia. These cells are easier to isolate, culture, and store compared to PHHs and liver slices [27].

In the development of the normal human embryo (Fig. 2), foregut endoderm gives rise to liver progenitor cells, which are differentiated into liver parenchymal cells (PCs) such as cholangiocytes and hepatocytes.

Other non-parenchymal cells are formed from mesoderm.

To simulate this process, human skin fibroblasts are first de-differentiated into iPSCs using growth factors Oct 4 and Sox 2 (Fig. 3) [28]. The iPSCs are then differentiated into foregut cells using the growth factors, FGF, BMP, activin A, and GSK 3. Hepatocyte-like cells are further differentiated from iPSC-derived foregut cells using growth factors FGF, BMP, HGF and Wnt into PCs. iPSCs are differentiated into NPCs such as endothelial and kupffer cells using the growth factors FGF, TGFβ inhibitors, VEGF, and others [29]. Liver organoids are yielded in about 10 days from iPSCs, about 20 days from skin fibroblasts [10], [28].

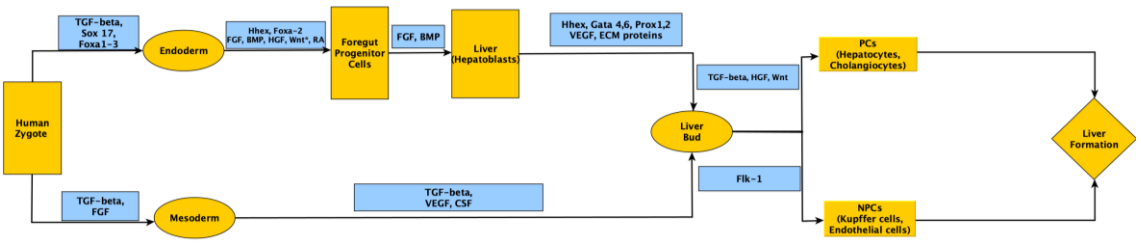

Fig. 2. *In vivo* formation of human liver.

Wnt* must be inhibited in endoderm to allow Hhex activation and foregut development, If secreted from mesoderm will suppress foregut development.

*Unrelated details are not shown in the figure [30]-[33].

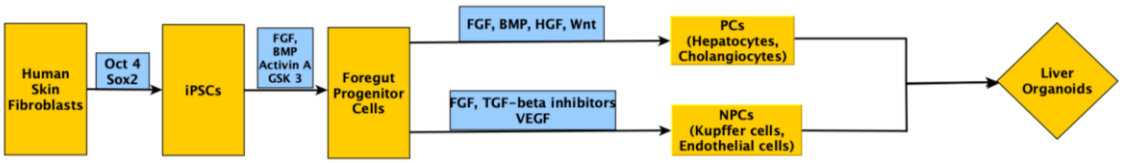

Fig. 3. *In vitro* formation of liver organoids [28], [29].

4.1. Liver Organoid Systems

The 3D liver organoid systems can comprise extracellular matrix (ECM) scaffolds, spheroid organoids, bioreactors, and microfluidic devices. Matrigel is the most commonly used media, which is made up of protein mixtures that are to represent the ECM *in vivo* that supports cell structures and transmits signals to cells [34]. The culture systems can be realized through either static or perfusion systems [35]. The static system is easier to implement, where the media surrounds the organoids and is changed every given period. The perfusion system, which is built in microfluidic devices and bioreactors, has media perfuse through the organoids in order to mimic blood flow *in vivo*. This is important for xenobiotics metabolism as blood flow creates a zonal differentiation of oxygen and nutrients [36]. One key drawback of traditional cell cultures is they are not able to include the oxygen gradient and nutrients cycling available with perfusion, yet 3D liver organoids with perfusion systems are more similar to *in vivo* liver, thereby enhancing human relevance for *in vitro* toxicity testing.

4.2. Hepatic Functionality

The functionality of liver organoids is proven to be better than conventional models by the evaluation of albumin secretion, CYP 450 family enzyme inducibility, urea production, and lifespan. Hepatic marker genes such as albumin (ALB), CYP2C9, and CYP7A1 are shown to be upregulated, with decreased undifferentiated state gene expression progressively such as alpha-fetoprotein (AFP) [10]. In addition, the albumin secretion capacity of iPSC-derived liver organoids, as well as CYP2C9 activities upon induction by rifampicin, are comparable to PHHs from the primary liver [10]. The liver organoids can generally survive about 30 days

with maintained albumin, urea, and CYP enzyme levels, which allow for acute and chronic toxicity studies [10], [11]. The hepatic functionalities of iPSC-derived liver organoids are highly relevant to *in vivo* human liver, which underscores their greater potential for drug toxicity prediction by recreating a human liver microenvironment.

Transcriptomic data was also analyzed to observe the protein expression level of the iPSC-derived liver organoids. Genes involved in xenobiotic metabolic processes are highly upregulated in iPSC-derived liver organoids [9]. Various hepatic cell types were revealed including stellate cells, endothelial cells, and cholangiocytes. Gene expressions of NPCs are nearly identical to PHHs, and those of PCs are similar to PHHs [10].

4.3. Maturation Status of Hepatocytes

The maturation of PCs is closely related to the presence of NPCs, as shown by several studies. NPCs are responsible for secreting growth factors (e.g. BMPs, TGFβ, FGF) that are important for PC proliferation. Meanwhile, the elevation of ALB and TDO2 gene expression, the reduction in AFP secretion, and the increase in the secretion of albumin mark the hepatocytes maturation in the NPCs co-culture system [37]. The maturation status of PCs determines the accuracy of liver organoids for toxicity prediction as the hepatocytes contain major CYP enzymes for drug metabolism.

Jin *et al.* tested the maturation status of liver organoids in four groups: 1) PCs only in static media, 2) PCs only in microfluidic devices, 3) 3D co-culture liver system with NPCs (i.e., Endothelial cells) in static media and 4) 3D co-culture liver system with NPCs (i.e., Endothelial cells) in microfluidic devices. The albumin secretion and urea synthesis are maintained and are enhanced with fluid flow. In addition, urea, albumin, and CYP3A4 activities are observed to be highest in co-culture system in a microfluidic device when evaluated in four systems described above [9].

Another research group Wang *et al.* obtained similar results with the microfluidic 3D liver model better at both maintaining hepatic functions and enhancing iPSC-derived synthetic endoderm differentiation into various cell types [11]. These findings demonstrate co-culture with NPCs is proven to generate higher organoid functionality and vascularized liver organoid systems are of the same significance to resemble *in vivo* liver. Therefore, it is expected that with the co-culture of NPCs in a fluid flow media, iPSC-derived liver organoids can further differentiate and mature, thus enhancing DILI prediction.

However, there are research indicating that half of the iPSC-derived liver organoid cells are immature due to their immature transcriptome signatures in comparison to adult liver tissue derived organoids [9], [10]. Despite this, the iPSC-derived liver organoids are still useful models as they are able to predict DILI and/or IDILI, shown by several groups Shinozawa *et al.*, Wang *et al.*, and Jin *et al.* It is true that the more differentiated organoids can better resemble human liver, but iPSC-derived liver organoids are functional tools as long as they can accurately detect IDILI and/or DILI.

4.4. High Throughput Testing

iPSC-derived organoids are proposed as a viable drug testing tool, supported by their potential for high throughput testing, which is essential to achieve when introducing this tool for drug screening.

4.4.1. Intrinsic DILI

Acetaminophen accounts for the majority of intrinsic DILI in the U.S [38]. The microplate array format of the microfluidic device used by Jin *et al.* enables chambers that hold liver organoids to be fluidically connected by microchannels, which allows continuous perfusion without an external pump. This device also demonstrates the possibility of detecting toxicity caused by acetaminophen (APAP), showing a concentration-dependent decrease in glutathione (GSH) and an increase in reactive oxygen species (ROS) [9].

4.4.2. IDILI

Previous studies of iPSC-derived liver organoids have successfully detected DILI in 206 marketed drugs by culturing organoids in a static system using 384-well plates with 238 drug treatments in different doses, including drugs identified to cause IDILI such as Troglitazone, Bosentan, and more [10].

Both the Jin *et al.* and Shinozawa *et al.* studies open possibilities of high throughput drug screening performed on liver organoids in the future. As it will take substantial time to shift toxicity testing to predominantly *in vitro*, iPSC-derived organoids are a strong candidate for a toxicity testing platform that can yield promising and clinically significant information on hepatotoxicity.

4.5. DILI Biomarkers

Some DILI biomarkers that are looked at in iPSC-derived liver organoids include mitochondrial dysfunction, cell viability, and cholestatic functions. Jin *et al.* quantified dose dependent APAP toxicity by measuring the level of GSH and ROS. More directly, acute APAP toxicity was assessed by cell viability over 48 hours by CCK-8 assay [11]. Additionally, mitochondrial dysfunctions and bile salt export pump (BSEP) inhibition, which are two known causes of DILI, were monitored and measured in the high throughput liver organoid testing system by Shinozawa *et al.* Mitochondrial membrane potential (MMP) is measured by observing fluorescent dye aggregation in the cell as a marker for mitochondrial inhibition [39]. BSEP is examined by adding fluorescein diacetate or Cholyl-Lysyl-Fluorescein (CLF) to investigate bile acid transport into channel analogs of the liver organoids [10]. Shinozawa *et al.* were able to identify drugs that can cause cell death and cholestasis such as cyclosporine A, troglitazone, tolcapone, diclofenac, bosentan, and nefazodone within 24-72 hours [10].

Interestingly, the typical biomarkers for hepatocellular injury, serum alanine and aspartate transferase (ALT/AST) levels, were not tested in the three iPSC-derived liver organoid toxicity testing studies mentioned above. Since the FDA guidance suggests the discontinuation of a tested drug at 8 times the upper limit of ALT/AST level in phase 3 clinical trials, demonstration of ALT/AST elevation in liver organoids in preclinical testing is essential [40]. Future tests done for DILI and/or IDILI prediction would need to become more similar to human phase 3 clinical trials, where ALT/AST levels, bilirubin, and bile salts inhibitions are overseen [40]. Fig. 4 shows a flowchart of DILI and/or IDILI prediction by iPSC-derived liver organoids.

It should be emphasized the list of tests designed to measure hepatotoxicity described above (ALT/AST, albumin, urea, MMP and bile salts) is not meant to be comprehensive and it is always possible to add tests as needed for more accurate DILI and/or IDILI prediction.

5. Discussion

5.1. Feasibility of iPSC-Derived Liver Organoids for Hepatotoxicity Testing Exclusively

In this paper, we propose iPSC-derived liver organoids to replace current toxicity testing suites including *in vitro* cell culture and animal models given that iPSC-derived liver organoids recapitulate the human liver by producing comparable albumin, urea, and CYP enzyme levels to PHHs. There are a few considerations to implement this drug discovery tool in the market including reproducibility, quality control, similarities of liver organoids, price, and FDA approval.

Firstly, reproducible production of liver organoids from lot to lot is needed under standardized procedures. Reproducibility of the preparation of liver organoids ensures mass production of liver organoids from a single sample of iPSC retrieved. As demonstrated by Shinozawa *et al.*, their iPSCs are induced to foregut cells that can be frozen until the next use, which allows fewer extractions from donors.

In addition, liver organoids used in toxicity testing must all be made by the same procedure or toxicity

profiles of the same drug tested with different organoids may differ. For example, there must exist protocols for specifying growth factors added to the media at different stages of differentiation. Since current liver organoid systems comprise various types, and every research group grows their organoids in different media and settings (i.e., ECM scaffold, microfluidic devices, and bioreactors), further research needs to be done on determining which liver organoid system best represents the *in vivo* counterpart.

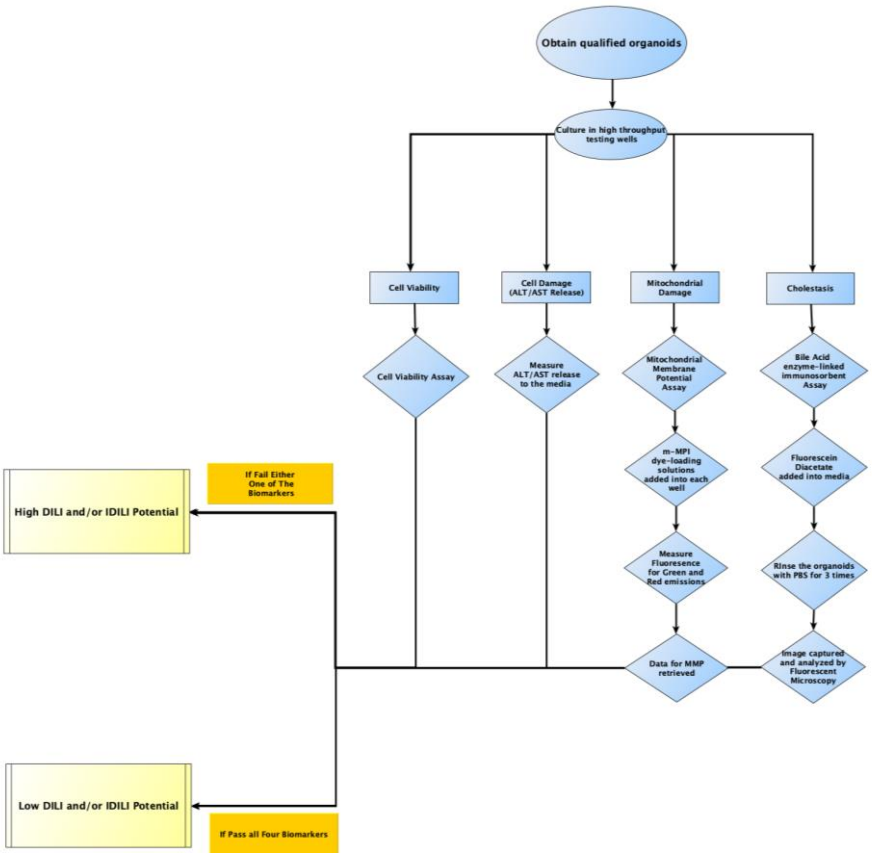

Fig. 4. Suggested workflow of iPSC-derived liver organoids testing. Mitochondrial potential indicator (m-MPI) *MMP can be visualized using Image-based MMP assay, which requires measuring fluorescence intensity at a different wavelength than the assay indicated in the graph [10], [26], [39].

Once a standard procedure for liver organoid preparation has been defined, the quality control measures should be implemented to ensure that all batches made by a single manufacturer are identical. Necessary quality control measures include assessments of liver functionalities such as urea production and defined specifications on protein expression levels such as albumin and CYP enzymes. The morphology of the liver organoids should also be taken into consideration when *in vitro* "liver" is built so that the orientations and the composition of the liver organoids well resemble the human liver [10]. Having a standardized protocol would ensure the accuracy of results as well as the reproducibility of drug safety assessment from lab to lab.

Lastly, liver organoids used in toxicity testing must be licensed by the FDA, manufactured under current good manufacturing practices (cGMP) and the manufacturing facility inspected by the FDA, as required. After the liver organoids have been approved by the FDA, it's crucial to introduce them at a low enough price for lab testing by pharmaceutical companies as an exclusive testing tool. A proposed procedures of making commercially available liver organoids is shown in Fig. 5.

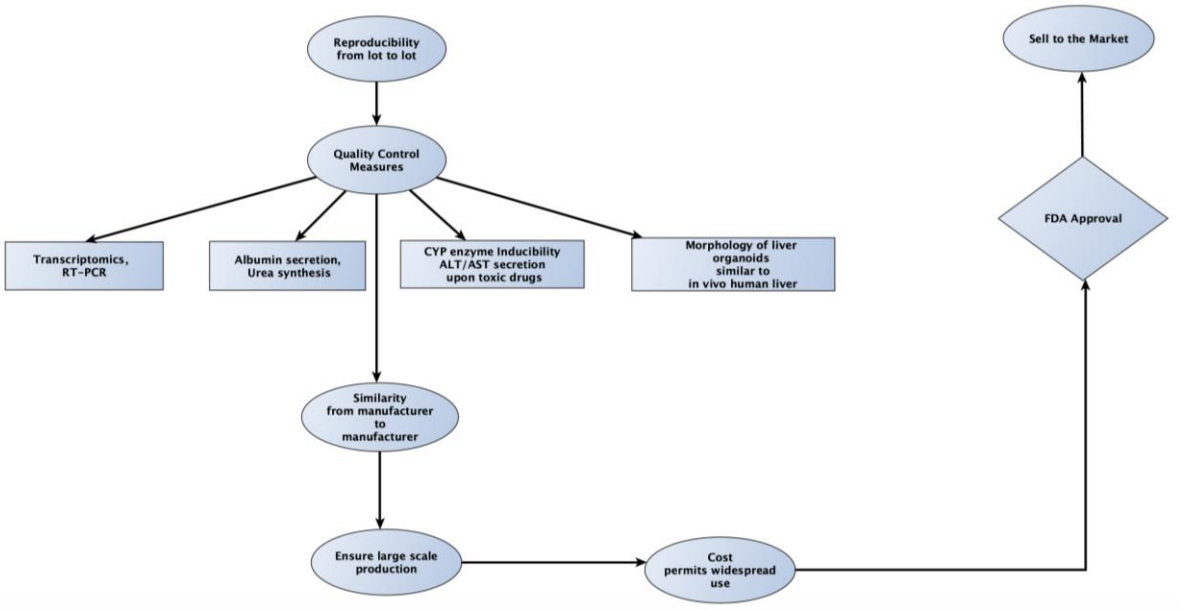

Fig. 5. Process of making iPSC-derived liver organoids commercially available.

It is early in the implementation process and the above obstacles would need to be overcome before iPSC-derived liver organoids are commercially available at low cost with high reproducibility. Nevertheless, this field is changing quickly, and current research on iPSC-derived organoids demonstrate promising results with respect to similarity to their *in vivo* counterpart as well as the possibility of high throughput drug testing. Although much work remains, rapid development of iPSCs as tools for toxicity testing is expected to continue.

5.2. Challenges for iPSC-Derived Organoids

Despite iPSC-derived liver organoids being superior to many *in vitro* cell cultures, there still exist drawbacks for this as a liver toxicity evaluation tool.

5.2.1. The prediction of IDILI

IDILI remains hard to predict and characterize as the symptoms do not appear until late phase 3 clinical trials or even post-market [41]. The famous story of drug withdrawal because of hepatotoxicity, Troglitazone, caused 135 cases of severe liver toxicity and six deaths in Japan and the US but remained on the US market for additional 2 years and 3 months after the drug was withdrawn from the market in the UK [42]. The prolonged period of Troglitazone remained on the market undoubtedly increased the accessibility to the drug and the risks related to it.

It has been theorized that IDILI is likely involved with the adaptive immune response, which is initiated by an innate response caused by reactive metabolites activated by CYP enzymes. However, mitochondrial injury, BSEP inhibition, unfolded protein response and oxidative stress have been proposed as causes of IDILI [41], [43]. This suggests that IDILI is multifactored, and the Shinozawa group, using liver organoids with no adaptive immune response, successfully detected IDILI in cyclosporine A, troglitazone, tolcapone, diclofenac, bosentan, and nefazodone based on mitochondrial dysfunction, BSEP inhibition, and reactive metabolites. This study indicates that it is possible to detect some IDILI using iPSC-derived liver organoids that are not immune-mediated. However, the Shinozawa group focused on cholestasis as the major cause of IDILI. Since cholestasis is not the only cause of IDILI, to detect a drug causing DILI more accurately through immune-mediated pathways, a substantial part of the adaptive immune system should be added (i.e., cytotoxic and T helper cells). This would add complexity to the liver organoids and will take a substantial

amount of time to accomplish. A great deal of effort is needed to show if iPSC-derived liver organoids are able to detect IDILI with an adaptive immune system involved.

5.2.2 Genetic variation and vulnerable populations

Furthermore, organoids derived from a single person do not take into account population variation (i.e., genetic and epigenetic) as well as additional environmental chemical exposures and the interactions of these with the drug of interest. This could lead to preclinical trials unable to predict hepatotoxicity. To tackle these problems, iPSCs from different donors should be tested and compared to identify individuals vulnerable to certain hepatotoxic drugs. It has also been shown that people with Hepatitis B (HBV) infection, obesity or nonalcoholic fatty liver disease might have different dose-dependent responses to hepatotoxic drugs [2], [10]. Simulations of the effects of lipid levels and HBV infection microenvironment need to be performed apart from standardized liver organoids tests to determine hepatotoxicity for vulnerable populations.

Additionally, more than 2,000 CYP enzyme mutations are reported, and within these, some polymorphisms have been described that can impact CYP enzyme activities, thereby affecting drug metabolism [44]. Since not all CYP enzymes are involved in drug metabolism, details should be investigated around CYP enzymes involved in metabolizing certain DILI drugs, for example, CYP2C9 for diclofenac, CYP2B6 for ticlopidine, and CYP3A in troglitazone [45], [46]. Inter-individual CYP variances also appear to be racial specific, which would require organoids from different races to be tested to account for CYP enzyme polymorphism.

To better account for variabilities within populations, organoids from different patients should be used in toxicity testing. However, there will need to be a standard to compare outcomes in different organoid systems to reach a conclusion of whether a drug possesses the potential of causing DILI. It is impossible to quantify the number of liver organoids that should be used for drug toxicity testing at present as it would depend on the advances of high throughput testing technology in the future.

It should be stressed that different liver organoids other than individuals with CYP enzyme polymorphism and liver diseases can be obtained for more accurate DILI and/or IDILI predictions if other vulnerable populations are identified.

Nonetheless, these are also the challenges current toxicity testing models face. The iPSC-derived liver organoids are not perfect as are many other toxicity testing models, However, a higher percentage of acute cases of DILI and at least some IDILI are shown to be predicted using this tool, which makes it superior to current toxicity testing tools in terms of higher human relevance, better results at predicting DILI and/or IDILI, and fewer tests done. This is very useful in preclinical trials to rule out more hepatotoxic drugs than existing methodologies could achieve.

6. Conclusion

Current toxicity testing models require multiple suites of tests on multiple test subjects, such as immortalized liver cells and live animals, yet fail to predict DILI and/or IDILI caused by the drug. iPSC-derived liver organoids greatly reduce the number of tests needed and meanwhile represent a testing model that is more similar to *in vivo* human liver, with the incorporation of major liver cell types, mimicry of blood circulation *in vivo*, and cell-cell interactions.

iPSC-derived liver organoids are evaluated to be functionally comparable to *in vivo* human liver through albumin secretion, urea synthesis, CYP 450 family enzyme inducibility as well as transcriptomic analysis on upregulation of genes responsible for xenobiotic metabolic processes.

The maturation status of hepatocytes in liver organoids has been examined with co-culture and under different media conditions. Studies show that a co-culture system with NPCs under a media with fluid flow

expresses fewer AFP genes and more ALB and TDO2 which marks the maturation of the hepatocytes [9], [37]. Although some studies suggest that some hepatocytes in iPSC-derived liver organoids are immature, in this paper I insist that as long as iPSC-derived liver organoids can accurately detect DILI and/or IDILI, they are a useful and reliable tool for toxicity testing [9], [10].

Although the detection of IDILI could be improved by inclusion of immune system components, iPSC derived liver organoids, as currently constituted, are a superior system for detection of acute DILI and IDILI than current testing methodologies. There still exist some barriers to produce iPSC-derived liver organoids in a reproducible, standardized, scalable, and cost-effective system. When these factors are satisfied and FDA approval is obtained, liver organoids can then be introduced for toxicity testing by pharmaceutical companies.

As has been shown, iPSC-derived liver organoids are a new strategy to solve existing issues that current toxicity testing cannot achieve including reducing the numbers of tests done, increasing human relevance of toxicity tests, and opening possibilities for high throughput and subchronic/chronic testing [8], [10], [14], [47]. Use of liver organoids in toxicity testing would therefore result in the elimination of more hepatotoxic drugs before clinical trials are begun, both saving money and reducing risk to vulnerable patients.

Conflict of Interest

The author declares no conflict of interest.

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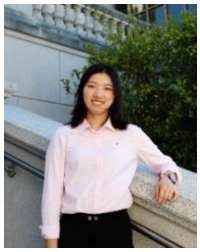

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