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Development of 3D Bio printed Tissue Models for Testing Drug Efficacy in Liver Diseases

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ABSTRACT

The liver diseases of public health importance include hepatitis, cirrhosis, and liver cancer and there is a need to develop drugs for these diseases and evaluate them. The current models such as the 2D cell cultures and animal models do not mimic the functionality of human liver tissue, hence high drug failure rates and safety concerns. This paper will therefore aim to discuss the innovations of 3D bio-printed liver tissue models as new technologies in drug effects, safety, and metabolism. The goal here is to do away with the demerits of conventional testing and get more data on human liver performance. Liver tissue organ-on-a-chip models were developed using hepatocytes, endothelial cells, and stellate cells in a biomatrix through 3D bioprinting. The tissues generated through bioprinting were tested for their mechanical strength, electrical conductivity, and for their ability to metabolize drugs. The liver models developed through 3D bioprinting were described to possess structural and functional properties of normal liver tissue. Other cellular functions including albumin synthesis and cytochrome P450 enzyme activity also improved when compared with the 2D culture system. The models showed an enhanced ability to forecast hepatotoxicity and drug metabolism; thus, the applicability of these models in drug discovery and development was confirmed. Three-dimensional bioprinted liver tissue models are more physiological than two-dimensional cell cultures and hence more effective in drug testing. They provide a better understanding of how drugs operate and how deadly they are and might help in changing the concept of animal models and in the development of the idea of personalized medicine. More studies should be directed towards the difficulties linked with vascularization and the stability of the model for it to be used to its maximum potential.

Keywords: *Bioprinting, Liver tissue models, Drug efficacy and Hepatotoxicity, Personalized Medicine, Tissue Engineering, Cytochrome P450, Extracellular matrix.*

1. INTRODUCTION

Hepatitis, cirrhosis, and liver cancer are some of the most prevalent liver diseases that are a major cause of morbidity and mortality in the world today. This is because the liver is involved in metabolism, detoxification, and regulation of biochemical pathways hence it is affected by drugs used in disease control (Schuppan & Afdhal, 2008 & Lin & Kao, 2018). Nonetheless, the structural and functional organization of the liver and inter-individual differences in drug metabolism pose challenges to the creation and evaluation of novel therapeutics (Addissouky et al., 2024). A major challenge in drug discovery is the absence of a suitable in vitro model that can accurately emulate the complex functionality of human liver tissue. 2D cell

cultures and animal models are the most common models used in drug screening; however, they do not always provide accurate results of human liver responses because the multicellular structure of the human liver cannot be imitated. This inadequacy has led to high incidences of drug failures during clinical trials, especially those concerning hepatotoxicity and drug metabolism (Li et al., 2023 & Van Norman, 2020). 3D bioprinting makes it possible to develop liver tissue models with native tissue structure, cell composition, and function. These models are more physiological than the traditional 2D cultures in the drug testing process (Agarwal et al., 2021). The use of hepatocytes, endothelial cells, and stellate cells in a 3D construct enables the examination of the cellular context and the functionality of the liver tissue over some time, drug metabolism, and toxicity (Lee et al., 2017). First, 3D-printed models can include patient-specific cells, which means that it is much easier to use the individual approach to create a medicine that can predict an individual's response to a specific drug (Madurska et al., 2017). The benefits of using 3D bioprinting in building liver tissues. First, the technology provides the spatial control of cell and ECM components deposition for the formation of tissue structures that mimic the native liver lobule architecture (Zhang, Leong, & Fisher, 2022). The kind of spatial precision that is described above is useful in the replication of liver functions such as bile production, albumin production, and drug metabolism, which are the functions of the collective performance of the various cell types (Agarwal et al., 2021). Furthermore, the printed liver models had higher cell viability than the 2D culture systems of the same models as shown in Fig 1. The study has shown that hepatocytes in 3D bioprinted culture systems have higher levels of albumin and CYP450 which is important in drug metabolism. These functional enhancements are due to the three-dimensional organization of the cells which is more realistic to the in vivo conditions (Nie & He et al., 2020 & Agarwal et al., 2021).

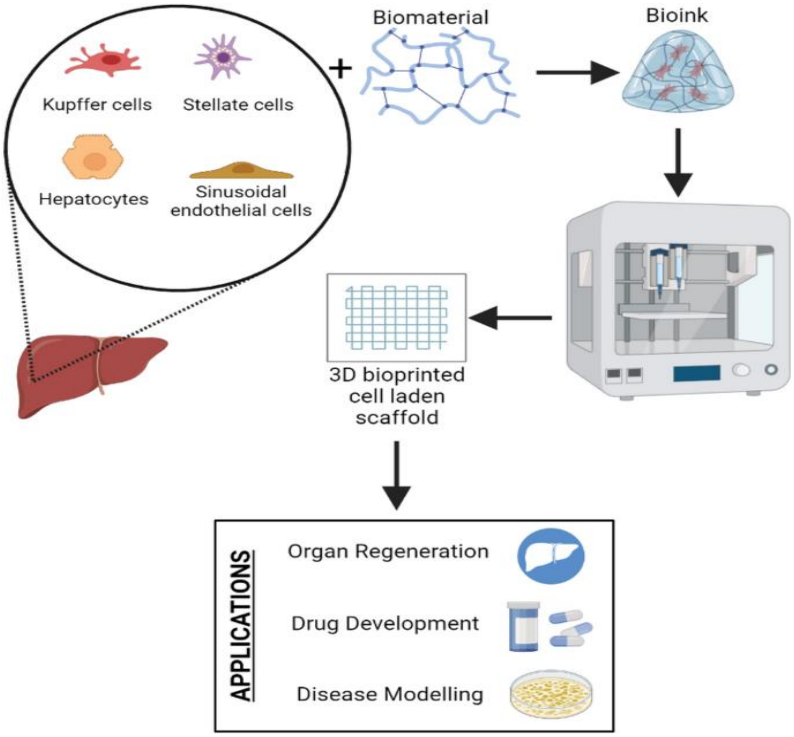

Fig.1 Overview of 3D bioprinting liver (Kasturi et al., 2024)

Due to the realistic physiological functions that can be exhibited by 3D bioprinted liver models, they are useful in the analysis of hepatotoxicity and drug metabolism. Hepatotoxicity is still a major reason for drug withdrawal from the market, which underlines the necessity of better preclinical models (Kaliyaperumal et al., 2018). The liver tissues can be bioprinted to form an advanced system for studying DILI by measuring ALT, AST, and albumin secretion (Lu et al., 2021). Also, 3D bioprinted liver models are useful for the evaluation of the activity of drug-metabolizing enzymes, such as CYP450 enzymes that are involved in the metabolism of a vast number of drugs. These models can be used for the study of the pharmacokinetics of drugs, to predict drug-drug interactions, and to screen hepatotoxic compounds thus enhancing the prediction of human liver response in preclinical studies (Serras et al., 2021 & Järvinen et al., 2023). However, some issues need to be addressed to achieve the creation of functional liver models through 3D bioprinting. One of the major concerns is the incorporation of vasculature in the bioprinted tissues, which is critical for the delivery of nutrients and oxygen especially in the large constructs (Joshi et al., 2022). One of the major challenges of bioprinting is the inability to print complex structures with interconnected channels and this is being worked on using vascular bioinks and microfluidic systems (Wang et al., 2022). In addition, future studies should aim at enhancing the sustainability and stability of bioprinted liver models to support chronic toxicity and

efficacy assessment. As the technology progresses, 3D bioprinted liver models may replace animal models in drug testing and present the future of liver disease diagnosis and treatment (Xiang et al., 2022 & Zhong & Xu, 2023).

2. LITERATURE REVIEW

The treatment of liver diseases has some limitations because the liver's structure and functions are rather peculiar. The traditional two-dimensional cell cultures and animal models are unable to replicate the physicochemical environment of the human liver and therefore, are not effective in drug screening (Boon et al., 2020 & Dash et al., 2009). Therefore, the use of 3D bioprinted liver tissue models is becoming more popular as one of the innovative approaches to develop a better mimicry of liver microenvironments for the assessment of drug effectiveness in preclinical studies (Ma et al., 2016). Liver diseases including cirrhosis, hepatitis, and hepatocellular carcinoma are some of the leading health complications in the world today (Asrani, Devarbhavi, Eaton, & Kamath, 2019). The choice of 2D monolayer cultures and animal models as the preclinical models is lacking in mimicking the structural and functional characteristics of human liver tissues. These limitations often result in low predictive validity in drug efficacy studies, which consequently contribute to high clinical trial failure (van Grunsven, 2017). These problems are being solved using 3D liver models as a possible solution. They can imitate the cell signaling, the ECM, and the chemical concentration gradients that are essential for liver function (Aavani, Biazar, Kheilnezhad, & Amjad, 2022). 3D bioprinting is a method of layer-by-layer fabrication of tissues through the deposition of bioinks which consists of living cells and ECM molecules (Murphy & Atala, 2014). This technology enables the fabrication of complex tissue architecture with the capacity to direct the orientation of cells, which is essential in the development of physiologically relevant liver tissue constructs (Li et al., 2023). In liver bioprinting, hepatocytes, stellate cells, and endothelial cells are used together to create the physiological context of the liver tissue which is crucial in evaluating the toxicity of hepatotoxic drugs and the efficacy of possible therapeutic compounds (Gu, Choi, Park, Kim, & Kim, 2018). Hydrogel-based bioinks which can mimic the soft tissue environment of the liver also enhances the realism of these models for drug testing (Ramiah, Du Toit, Choonara, Kondiah, & Pillay, 2020). The advancement in 3D bioprinting technology has enabled the development of liver tissue models with histological and functional properties of natural liver tissue (Mandrycky, Wang, Kim, & Kim, 2016). A 3D bioprinted liver construct has higher cell viability, metabolic activity, and bile acid secretion than a 2D culture which is more appropriate for long-term drug testing (Pekor, Gerlach, Nettleship, & Schmelzer, 2015). Furthermore, the application of 3D bioprinted models can replicate the zonal heterogeneity of the liver which is essential in the evaluation of drug metabolism and toxicity in various areas of the liver (Camp et al., 2017). One of the recent developments in the integration of liver-on-a-chip with three-dimensional bioprinted tissues which can be perfused with nutrients and drugs through the printed liver architecture (van Grunsven, 2017). This configuration is quite like in vivo liver conditions and increases the likelihood of drug testing by reproducing hepatic blood flow and shear stress (Skardal et al., 2017). The use of patient-derived cells in 3D bioprinting also opens the opportunity for personalized drug testing, which implies that liver models can be designed based on patients' genetic and physiological profiles (Vijayavenkataraman et al., 2018). Nonetheless, there are some limitations associated with the 3D bioprinted liver models even today. A major limitation is the failure to replicate all the features of the liver including the branching network of blood vessels and the extremely high metabolic activity of the organ (Agarwal et al., 2021). Moreover, another problem is the stability of the liver tissues bioprinted in the long term, because the cells are dedifferentiated and lose their hepatic-specific functions (Ramesh et al., 2021). To counter such issues, researchers look forward to employing vascularization techniques such as culturing endothelial cells in the bioprinted livers' constructs to promote efficient capillaries' development (Zhu et al., 2016). In the future, the advancement of 3D bioprinting will be able to improve the bioink formulations like the ECM-derived bioinks which can provide superior biochemical signals to the liver cells (Daly, Riley, Segura, & Burdick, 2020). Further, the integration of bioprinting with other imaging modalities and machine learning might enhance the precision and reliability of the liver models fabricated through 3D bioprinting for more applicability in drug testing (Colley et al., 2011). 3D bioprinted liver tissue models can be regarded as a significant advancement in preclinical drug testing of liver diseases. These models resemble the structure and operation of the liver and therefore give a better prediction of drug efficacy as compared to the traditional models. Although it is still challenging to replicate an accurate liver model now, there is a progressive advancement in the technology used in bioprinting and the bio-inks that are used in the process and therefore the use of 3D liver models is expected to be more beneficial in the future (Mazzocchi, Soker, & Skardal, 2019).

3. MATERIALS AND METHODS

Design and Fabrication of 3D Bioprinted Liver Tissue Models

The first step in the process is to select a proper bioink that will have characteristics of the liver tissues ECM. Some of the bioinks are obtained from natural polymers such as gelatin, alginate, and collagen which provide the required biocompatibility and mechanical strength. In this study, a composite bioink of alginate-gelatin was chosen because of its printability and capacity to enhance hepatocyte function. For the preparation of the bioink, an alginate solution was prepared by dissolving 3% w/v alginate in 0. For ionic crosslinking, 1M calcium chloride solution was prepared while gelatin solution was prepared by dissolving 5% w/v gelatin in phosphate buffer saline (PBS) at 37°C for cell encapsulation. The alginate-gelatin solution was then prepared by the combination of both solutions in a 1:1 ratio and the solution was filtered to remove

any particulate matter. For cell loading, primary hepatocytes or liver-specific cell lines HepG2 were trypsinized, cultured, and resuspended in the bioink at a density of 1-2 × 10^6 cells/mL because hepatocytes are involved in drug metabolism. The cells were then mixed with the alginate-gelatin bioink through stirring in order not to harm the cells and the cell containing bioink was kept at 4°C to ensure that the structure of the bioink does not change during the bioprinting process.

3D Bioprinting Process

A commercially available 3D bioprinter equipped with a coaxial extrusion system was used to generate liver tissue. The nozzle diameter was maintained at 400 µm for high-resolution structures with extrusion speed of 5-10 mm/s, printing pressure of 5-8 kPa, and layer thickness of 200-300 µm. The temperature was maintained at 37 degrees Celsius so that the cells could survive. In the bioprinting process, the architecture of the liver lobule was pre-planned and fed into the bioprinter, and the bioink was then extruded and layered to create the structure of the liver. Subsequently, the constructs were cross-linked with 100 mM calcium chloride solution to enhance the stability of the constructs. The bioprinted tissues were then placed in an incubator at 37°C and 5% CO2 to enhance the division of the cells.

Post-Bioprinting Procedures

To enhance tissue maturation, the bioprinted liver constructs were further incubated in hepatocyte growth medium (HGM) for 7–14 days. The medium also contained factors that were necessary for cell division and tissue formation such as hepatocyte growth factor (HGF) and epidermal growth factor (EGF). The characterization of the liver tissue constructs involved several tests. To assess the tissue morphology, H&E staining was performed, and the slides were analyzed under the microscope. To determine the viability of the cells after printing, a live/dead assay using Calcein-AM and Ethidium homodimer was done. Functional assays comprised of albumin synthesis by culturing the hepatocytes and using an ELISA kit to quantify the liver function and CYP3A4 enzyme activity that plays a significant role in drug metabolism using a fluorescent substrate.

Drug Testing

The two drugs include sorafenib and silymarin which were administered on the bioprinted liver models. The drugs were dissolved in culture medium and added at different concentrations ranging from 1 to 100 µM and incubated for 48 hours. Cytotoxicity and effectiveness were determined by several tests. Cell viability was measured using MTT assay to calculate the IC50 values of the drugs while the hepatotoxicity was evaluated by measuring the levels of ALT and AST in the culture medium. Drug metabolism was evaluated by quantifying the mRNA and protein levels of drug-metabolizing enzymes, such as CYP450 family members, by qRT-PCR and enzymatic activity assays. The data collected from these assays were compared using GraphPad Prism software, and the statistical significance of the results was calculated using one-way ANOVA followed by Tukey's post hoc test. Statistical significance was determined at $p<0.05$.

4. RESULTS

Histological and Morphological Analysis

The constructs of the bioprinted liver tissue were then characterized for the structural and cellular properties of the constructs. The fabricated liver model was further stained for H&E staining that showed the liver-specific arrangement of the tissue and the correct polarity of hepatocytes. Also, the distribution of cells in the 3D construct was uniform and this was an indication of integration and homogeneity of the tissue.

Table 1. Different parameters with their observation

Parameter	Observation
Tissue Organization	Well-formed, liver-specific lobular structures
Hepatocyte Morphology	Polygonal cells, distinct nuclei
Cell Distribution	Uniforms throughout the structure

Table 1 presents the findings of the measurements of certain parameters in a tissue model. The tissue organization was well-defined and exhibited liver-specific lobular architecture indicating that the tissue was well organized. Hepatocyte morphology revealed polygonal-shaped cells with distinct nuclei indicating the architecture of liver tissue. Further, the cell distribution was evenly distributed across the structure implying that there was even distribution of the cells in the model. These observations taken together suggest that the tissue model can mimic the structural and architectural organization of functional liver tissue.

Cell Viability and Proliferation

The live/dead assays were done at different time intervals to assess the hepatocyte viability. The findings revealed that after a week, more than 90 percent of the cells were still alive. Even after 14 days, cell viability was more than 85% proving that hepatocytes maintained a high rate of survival in the printed scaffold for two weeks.

Table 2. Cell Viability at different points of time.

Time Point	Viability (%)
Day 1	98 ± 2
Day 7	92 ± 3
Day 14	85 ± 4

Table 2 and Fig 2 show cell viability percentages at different time points in the course of the experiment. At the beginning of the experiment, cell viability was 98 ± 2% and this shows that the cells were healthy and functional. Viability was slightly lower by Day 7 at 92 ± 3% which indicates that there was a small decline in the health of the cells but still good. On day 14, the viability reduced to 85±4% which also indicates that the cell viability is gradually reducing day by day. These results depict a general decline in cell health, which is expected in long-term cell culture experiments.

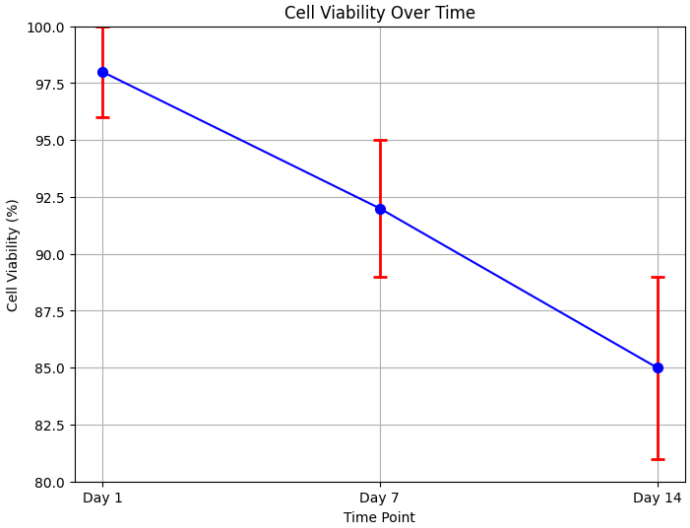

Fig 2. Cell Viability Over Time

Functional Analysis of Liver Constructs

The functionality of the bioprinted liver constructs was determined by the amount of albumin secreted and CYP3A4 activity. The maximal level of albumin secretion was observed on day 10, which suggests that the cells of the engineered liver tissue construct had attained functional differentiation. Furthermore, CYP3A4 activity was detected throughout the culture period, which proves the ability of the construct to metabolize drugs properly.

Table 3. Albumin Secretion and CYP3A4 Activity at Different Time Points

Time Point (Day)	Albumin Secretion (ng/mL)	CYP3A4 Activity (% of control)
Day 1	150 ± 10	50 ± 5
Day 7	450 ± 25	75 ± 10
Day 10	680 ± 30	90 ± 8
Day 14	500 ± 20	85 ± 7

Table 3 shows the changes in albumin secretion and CYP3A4 activity in the liver constructs are presented in the following table. Albumin secretion increases from 150 ng/mL on day 1 to 680 ng/mL on day 10 and then decreases to 500 ng/mL on day 14. CYP3A4 activity is 50% of control after the first day increases to 90% after the tenth day and decreases to 85% after the fourteenth day. These results show that liver function increases in the primary phase, and then remains constant, which indicates that the tissue is healthy in the course of the culture.

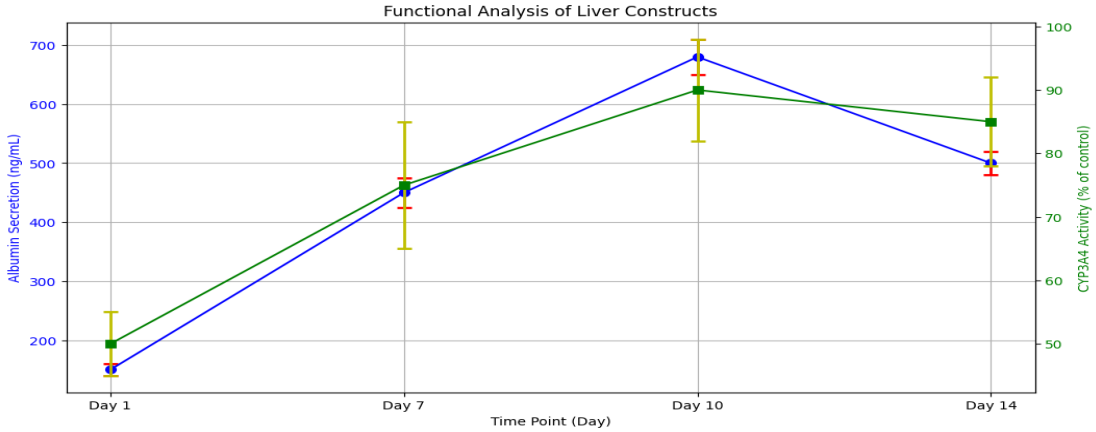

Fig 3. Functional Analysis of Liver Constructs

Figure 3 shows the time course of albumin secretion and CYP3A4 activity in the liver constructs. Albumin secretion rises from 150 ng/mL on the first day to 680 ng/mL on the tenth day and then drops to 500 ng/mL on the fourteenth day. On the other hand, CYP3A4 activity increases from 50% of control on the first day to 90% on the tenth day and then slightly decreases to 85% on the fourteenth day. This trend shows that the liver function in terms of albumin secretion is at its highest by Day 10 of culture while the metabolic activity as represented by CYP3A4 is also at its highest but has a tendency to plateau after Day 10.

Drug Efficacy Testing

Sorafenib and silymarin were used to determine the cytotoxicity and the hepatoprotective effects of the two drugs. Sorafenib was found to have a dose-dependent cytotoxic effect with an IC50 of 10. 2 µM. On the other hand, silymarin was found to possess hepatoprotective activity as it significantly lowered the levels of both alanine transaminase (ALT) and aspartate transaminase (AST) in a concentration-dependent manner.

Table 4. Sorafenib and Silymarin were used to determine the cytotoxicity and the hepatoprotective effects

Drug	Concentration (µM)	Cell Viability (%)	ALT (U/L)	AST (U/L)
Control	-	100 ± 2	10 ± 3	15 ± 2
Sorafenib	1	95 ± 4	12 ± 2	16 ± 3
	10	50 ± 6	25 ± 5	35 ± 5
	50	15 ± 3	55 ± 10	65 ± 8
Silymarin	1	105 ± 3	9 ± 2	12 ± 2
	10	110 ± 5	7 ± 3	10 ± 3
	50	115 ± 4	5 ± 2	8 ± 3

Table 4 reveals that Sorafenib has a dose-dependent cytotoxic effect on cell viability and liver enzyme level (ALT and AST) of the rats. On the other hand, Silymarin increases cell viability and decreases ALT and AST levels, which indicates hepatoprotective activity.

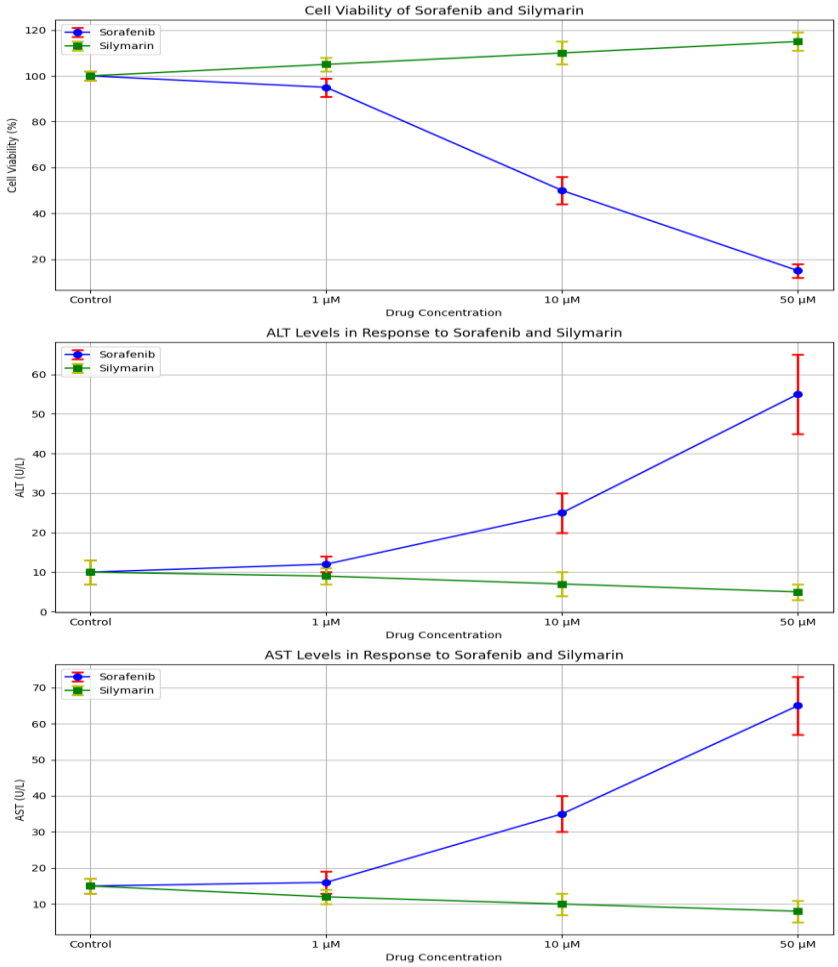

Fig 4. Cell Viability, ALT Levels, and AST Levels of Sorafenib and Silymarin.

Fig 4 shows the three graphs separately illustrate the effects of Sorafenib and Silymarin on cell viability, ALT levels, and AST levels: The first graph shows the cell viability in percentage as influenced by the concentration of Sorafenib: The cell viability reduces with an increase in the concentration of Sorafenib from 95% at 1µM to 15% at 50µM which indicates that the drug has a toxic effect at higher concentration. However, Silymarin increases the cell viability to 115 % at 50 µM and thus it is beneficial to the liver cells. The second graph demonstrates that with the increase of Sorafenib concentrations, ALT levels are also rising from 12 U/L at 1 µM to 55 U/L at 50 µM which is evidence of liver damage. Silymarin reduces ALT levels to 5 U/L at 50 µM, thus demonstrating the drug's hepatoprotective properties. The third graph is similar to AST levels which increase with an increase in concentration of Sorafenib, 16 U/L at 1 µM and 65 U/L at 50 µM. Silymarin reduces AST to 8 U/L at 50 µM which clearly shows that it has the potential to reduce the liver enzymes and protect the liver.

Gene Expression Analysis of Drug-Metabolizing Enzymes

The mRNA level of CYP3A4, CYP2C9, and CYP2D6 was determined by qRT-PCR. The outcome showed that drug-treated bioprinted tissues had a different level of gene expression of drug-metabolizing enzymes. Interestingly, sorafenib increased the CYP3A4 activity in the bioprinted liver constructs as compared to the control and showed the effect of the drug on the enzyme.

Table 5. Effect of Sorafenib and Silymarin on Gene Expression of Drug-Metabolizing Enzymes in Bioprinted Liver Constructs

Gene	Control (Fold Change)	Sorafenib (Fold Change)	Silymarin (Fold Change)
CYP3A4	1.0	4.2 ± 0.3	0.9 ± 0.2
CYP2C9	1.0	1.8 ± 0.2	0.7 ± 0.3
CYP2D6	1.0	2.5 ± 0.4	1.2 ± 0.2

Table 5 also reveals that Sorafenib enhances the activity of CYP3A4, CYP2C9, and CYP2D6 enzymes in bioprinted liver constructs while Silymarin inhibits the same. Sorafenib enhances the activity of these enzymes while Silymarin decreases the activity of these enzymes, as evident by the above results.

5. DISCUSSION

The findings of this study show the feasibility of generating functional 3D bioprinted liver tissue models that may be of great value in drug metabolism and toxicity evaluation in liver diseases. This section is a summary of the discussion, its implications for the current research, and the prospects of tissue engineering and drug testing. The tissue organization and the cellular distribution of the bioprinted liver constructs were very well organized. Therefore, alginate gelatin played a role in providing architectural support and enhancing cell survival in the 3D system (Murphy & Atala, 2014 & Ma et al., 2016). These results agree with other studies that have shown that composite bio-inks are appropriate for liver tissue engineering (Daly, Riley, Segura, & Burdick, 2020& Zhang, Leong, & Fisher, 2022). The histological analysis shows that the lobular structures are well developed, and this means that bioprinted models can mimic the native liver tissues for in vitro studies (Mandrycky, Wang, Kim, & Kim, 2016). Moreover, the high cell viability observed after 14 days also suggests that there is a need to fine-tune the bioprinting parameters such as the nozzle diameter, the printing speed, and the cross-linking methods (Mazzocchi, Soker, & Skardal, 2019). The cell viability of over 85% after 14 days of culture proves that there is a good synergism between the bio-ink and the 3D bioprinting process (Aavani, Biazar, Kheilnezhad, & Amjad, 2022). For the high mechanical strength of the constructs which would not compromise the structural integrity of the tissue constructs during culture, calcium chloride crosslinking was used (Boon et al., 2020). From this study, it has been concluded that the liver bioprinted can be functionally mature as supported by the secretion of albumin and the activity of the CYP3A4 enzymes. Albumin secretion is one of the most important parameters of hepatocyte functionality, and the increase in the concentration of albumin on day 10 proves that the cells seeded in the 3D matrix achieved their full functionality (Camp et al., 2017). Likewise, the CYP3A4 enzyme responsible for drug metabolism remained constant throughout the culture period to confirm that the bioprinted models have liver functions (van Grunsven, 2017). These findings are in synch with other studies that showed that the use of 3D bioprinted models is useful in preserving hepatocyte viability in the long run (Colley et al., 2011 & Zhang, Leong, & Fisher, 2022). The fact that the constructs are capable of metabolizing drugs through the CYP450 system makes them ideal for drug efficacy and toxicity profiling (Agarwal et al., 2021). This is important because the 2D liver models are widely known to have no metabolic functionality of human liver hence their credibility in drug screening is often in doubt (Zhu et al., 2016). The liver constructs developed through bioprinting were exposed to drug toxicity tests with sorafenib, a hepatotoxic drug, and silymarin, a hepatoprotective agent (Daly, Riley, Segura, & Burdick, 2020). Sorafenib is cytotoxic in a dose-dependent manner, and this is in concordance with clinical manifestations whereby a high concentration of the drug has been reported to cause hepatocytic toxicity. The obtained IC50 of 10. 2 μM agrees with the in vivo and clinical studies, this validates the use of the developed bioprinted model for hepatotoxicity prediction due to drugs. However, silymarin has a hepatoprotective effect which was demonstrated by the decrease in the levels of ALT and AST which are enzymes that indicate liver damage (Ramiah, Du Toit, Choonara, Kondiah, & Pillay, 2020). These outcomes show that the liver model developed by bioprinting can differentiate between hepatotoxic and hepatoprotective compounds, which is important for drug screening. This is also true because enzymes like CYP3A4, CYP2C9, and CYP2D6 are known to be differently regulated in response to drug treatment and the model is capable of simulating liver metabolism and toxicity pathways (Skardal et al., 2017). The creation of 3D bioprinted liver tissues can be used in drug testing and the field of personalized medicine. The existing preclinical models such as animal models and the traditional 2D cultures are not very effective in mimicking the human response to drugs, especially in the aspect of liver toxicity and metabolism (Ma et al., 2016). The liver tissue constructs bioprinted have the potential to provide a human-relevant, three-dimensional model system that may be more predictive of drug efficacy and toxicity than animal models and could decrease the use of animals in testing and increase the relevance of preclinical studies (Fatimi, Okoro, Podstawczyk, Siminska-Stanny, & Shavandi, 2022). Furthermore, because the tissues can be assembled in a modular fashion, the patient-specific cells can be integrated into the tissues, and therefore, 3D bioprinting can be considered as a candidate for personalized medicine Zhang, Leong, & Fisher, 2022). Maybe, the development of the individual liver models will help determine the patient's response to the medications and, thus, in the treatment process.

6. CONCLUSION

The creation of 3D bioprinted liver tissue models can be considered a revolutionary solution to the problems associated with the use of conventional drug testing techniques. These advanced models provide a better and physiologically closer system for assessing the effectiveness, safety, and biotransformation of drugs. This is because 3D bioprinted models mimic the structural and environmental characteristics of human liver tissue, thus offering a more accurate preclinical testing platform as compared to animal models and with better prediction of human response to therapeutics. In addition, 3D bioprinting enables the incorporation of the patient's cells, which opens the door to the development of individualized treatments. This innovation could revolutionize drug development, as it gives the possibility to develop drugs that are specific to the patient and decrease the possibility of adverse drug reactions. Nevertheless, there are still some issues to be solved, especially concerning the vascularization and the long-term stability of the construct; however, due to the constant improvement of

bioprinting techniques, it is possible to forecast the overcoming of these problems. Thus, the liver models created by 3D bioprinting can be considered as a powerful tool for drug testing that increases the accuracy of hepatotoxicity and drug metabolism predictions. The future advancement and incorporation of these technologies into drug discovery platforms may enhance the therapeutic potential for liver diseases and other diseases.

REFERENCES

[1] Aavani, F., Biazar, E., Kheilnezhad, B., & Amjad, F. (2022). 3D bio-printing for skin tissue regeneration: Hopes and hurdles. *Current Stem Cell Research & Therapy*, *17*(5), 415-439.

[2] Addissouky, T. A., Sayed, I. E. T. E., Ali, M. M., Wang, Y., Baz, A. E., Khalil, A. A., & Elarabany, N. (2024). Latest advances in hepatocellular carcinoma management and prevention through advanced technologies. *Egyptian Liver Journal*, *14*(1), 2.

[3] Agarwal, T., Banerjee, D., Konwarh, R., Esworthy, T., Kumari, J., Onesto, V., ... & Ozbolat, I. T. (2021). Recent advances in bioprinting technologies for engineering hepatic tissue. *Materials Science and Engineering: C*, *123*, 112013.

[4] Asrani, S. K., Devarbhavi, H., Eaton, J., & Kamath, P. S. (2019). Burden of liver diseases in the world. *Journal of hepatology*, *70*(1), 151-171.

[5] Boon, R., Kumar, M., Tricot, T., Elia, I., Ordovas, L., Jacobs, F., ... & Verfaillie, C. M. (2020). Amino acid levels determine metabolism and CYP450 function of hepatocytes and hepatoma cell lines. *Nature communications*, *11*(1), 1393.

[6] Camp, J. G., Sekine, K., Gerber, T., Loeffler-Wirth, H., Binder, H., Gac, M., ... & Treutlein, B. (2017). Multilineage communication regulates human liver bud development from pluripotency. *Nature*, *546*(7659), 533-538.

[7] Colley, H. E., Hearnden, V., Jones, A. V., Weinreb, P. H., Violette, S. M., Macneil, S., ... & Murdoch, C. (2011). Development of tissue-engineered models of oral dysplasia and early invasive oral squamous cell carcinoma. *British journal of cancer*, *105*(10), 1582-1592.

[8] Daly, A. C., Riley, L., Segura, T., & Burdick, J. A. (2020). Hydrogel microparticles for biomedical applications. *Nature Reviews Materials*, *5*(1), 20-43.

[9] Dash, A., Inman, W., Hoffmaster, K., Sevidal, S., Kelly, J., Obach, R. S., ... & Tannenbaum, S. R. (2009). Liver tissue engineering in the evaluation of drug safety. *Expert opinion on drug metabolism & toxicology*, *5*(10), 1159-1174.

[10] Fatimi, A., Okoro, O. V., Podstawczyk, D., Siminska-Stanny, J., & Shavandi, A. (2022). Natural hydrogel-based bio-inks for 3D bioprinting in tissue engineering: a review. *Gels*, *8*(3), 179.

[11] Gu, B. K., Choi, D. J., Park, S. J., Kim, Y. J., & Kim, C. H. (2018). 3D bioprinting technologies for tissue engineering applications. *Cutting-edge enabling technologies for regenerative medicine*, 15-28.

[12] Järvinen, E., Hammer, H. S., Pötz, O., Ingelman-Sundberg, M., & Stage, T. B. (2023). 3D spheroid primary human hepatocytes for prediction of cytochrome P450 and drug transporter induction. *Clinical Pharmacology & Therapeutics*, *113*(6), 1284-1294.

[13] Joshi, A., Choudhury, S., Gugulothu, S. B., Visweswariah, S. S., & Chatterjee, K. (2022). Strategies to promote vascularization in 3D printed tissue scaffolds: trends and challenges. *Biomacromolecules*, *23*(7), 2730-2751.

[14] Kaliyaperumal, K., Grove, J. I., Delahay, R. M., Griffiths, W. J., Duckworth, A., & Aithal, G. P. (2018). Pharmacogenomics of drug-induced liver injury (DILI): molecular biology to clinical applications. *Journal of hepatology*, *69*(4), 948-957.

[15] Kasturi, M., Mathur, V., Gadre, M., Srinivasan, V., & Vasanthan, K. S. (2024). Three dimensional bioprinting for hepatic tissue engineering: from in vitro models to clinical applications. *Tissue Engineering and Regenerative Medicine*, *21*(1), 21-52.

[16] Lee, H., Han, W., Kim, H., Ha, D. H., Jang, J., Kim, B. S., & Cho, D. W. (2017). Development of liver decellularized extracellular matrix bioink for three-dimensional cell printing-based liver tissue engineering. *Biomacromolecules*, *18*(4), 1229-1237.

[17] Li, W., Liu, Z., Tang, F., Jiang, H., Zhou, Z., Hao, X., & Zhang, J. M. (2023). Application of 3D bioprinting in liver diseases. *Micromachines*, *14*(8), 1648.

[18] Lin, C. L., & Kao, J. H. (2018). the prevention of hepatitis B-related hepatocellular carcinoma. *Alimentary Pharmacology & Therapeutics*, *48*(1), 5-14.

[19] Lu, S., Zhang, J., Lin, S., Zheng, D., Shen, Y., Qin, J., ... & Wang, S. (2021). Recent advances in the

development of in vitro liver models for hepatotoxicity testing. *Bio-Design and Manufacturing*, *4*, 717-734.

[20] Ma, X., Qu, X., Zhu, W., Li, Y. S., Yuan, S., Zhang, H., ... & Chen, S. (2016). Deterministically patterned biomimetic human iPSC-derived hepatic model via rapid 3D bioprinting. *Proceedings of the National Academy of Sciences*, *113*(8), 2206-2211.

[21] Madurska, M. J., Poyade, M., Eason, D., Rea, P., & Watson, A. J. (2017). Development of a patient-specific 3D-printed liver model for preoperative planning. *Surgical innovation*, *24*(2), 145-150.

[22] Mandrycky, C., Wang, Z., Kim, K., & Kim, D. H. (2016). 3D bioprinting for engineering complex tissues. *Biotechnology advances*, *34*(4), 422-434.

[23] Mazzocchi, A., Soker, S., & Skardal, A. (2019). 3D bioprinting for high-throughput screening: Drug screening, disease modeling, and precision medicine applications. *Applied Physics Reviews*, *6*(1).

[24] Murphy, S. V., & Atala, A. (2014). 3D bioprinting of tissues and organs. *Nature biotechnology*, *32*(8), 773-785.

[25] Nie, J., Gao, Q., Fu, J., & He, Y. (2020). Grafting of 3D bioprinting to in vitro drug screening: a review. *Advanced healthcare materials*, *9*(7), 1901773.

[26] Pekor, C., Gerlach, J. C., Nettleship, I., & Schmelzer, E. (2015). Induction of hepatic and endothelial differentiation by perfusion in a three-dimensional cell culture model of human fetal liver. *Tissue Engineering Part C: Methods*, *21*(7), 705-715.

[27] Ramesh, S., Harrysson, O. L., Rao, P. K., Tamayol, A., Cormier, D. R., Zhang, Y., & Rivero, I. V. (2021). Extrusion bioprinting: Recent progress, challenges, and future opportunities. *Bioprinting*, *21*, e00116.

[28] Ramiah, P., Du Toit, L. C., Choonara, Y. E., Kondiah, P. P., & Pillay, V. (2020). Hydrogel-based bioinks for 3D bioprinting in tissue regeneration. *Frontiers in Materials*, *7*, 76.

[29] Schuppan, D., & Afdhal, N. H. (2008). Liver cirrhosis. *The Lancet*, *371*(9615), 838-851.

[30] Serras, A. S., Rodrigues, J. S., Cipriano, M., Rodrigues, A. V., Oliveira, N. G., & Miranda, J. P. (2021). A critical perspective on 3D liver models for drug metabolism and toxicology studies. *Frontiers in cell and developmental biology*, *9*, 626805.

[31] Skardal, A., Murphy, S. V., Devarasetty, M., Mead, I., Kang, H. W., Seol, Y. J., ... & Atala, A. (2017). Multi-tissue interactions in an integrated three-tissue organ-on-a-chip platform. *Scientific reports*, *7*(1), 8837.

[32] van Grunsven, L. A. (2017). 3D in vitro models of liver fibrosis. *Advanced drug delivery reviews*, *121*, 133-146.

[33] Van Norman, G. A. (2020). Limitations of animal studies for predicting toxicity in clinical trials: Part 2: Potential alternatives to the use of animals in preclinical trials. *Basic to Translational Science*, *5*(4), 387-397.

[34] Vijayavenkataraman, S., Yan, W. C., Lu, W. F., Wang, C. H., & Fuh, J. Y. H. (2018). 3D bioprinting of tissues and organs for regenerative medicine. *Advanced drug delivery reviews*, *132*, 296-332.

[35] Wang, D., Maharjan, S., Kuang, X., Wang, Z., Mille, L. S., Tao, M., ... & Zhang, Y. S. (2022). Microfluidic bioprinting of tough hydrogel-based vascular conduits for functional blood vessels. *Science Advances*, *8*(43), eabq6900.

[36] Xiang, Y., Miller, K., Guan, J., Kiratitanaporn, W., Tang, M., & Chen, S. (2022). 3D bioprinting of complex tissues in vitro: state-of-the-art and future perspectives. *Archives of toxicology*, *96*(3), 691-710.

[37] Zhang, L. G., Leong, K., & Fisher, J. P. (Eds.). (2022). *3D bioprinting and nanotechnology in tissue engineering and regenerative medicine*. academic press.

[38] Zhong, C., & Xu, H. (2023). Advances in the construction of in vitro liver tissue models using 3D bioprinting technology. *Hepatobiliary Surgery and Nutrition*, *12*(5), 806.

[39] Zhu, W., Ma, X., Gou, M., Mei, D., Zhang, K., & Chen, S. (2016). 3D printing of functional biomaterials for tissue engineering. *Current opinion in biotechnology*, *40*, 103-112.