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WHITE PAPER

Will Advances in Preclinical *In Vitro* Models Lower the Costs of Drug Development?

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THE PRICE TAG FOR DRUG DISCOVERY and development continues to rise with no signs of decreasing or even stabilizing. New drug development takes about 12 years to get to market at an average cost greater than $1.2 billion dollars.[1,2] Attrition rates for drugs from clinical trials to market vary depending on their categorization with NMEs (New Molecular Entities, generally small molecules and peptides) seemingly having more clinical failures than BLAs (Biologic License Applications which usually involve large, protein-based macromolecules).[3] With an overall average attrition rate of approximately 90%, for compounds moving from clinical trials to market, the process appears daunting at best given the demand of resources, costs, and time consumed in this effort.[4] How do we reduce the costs associated with this process? Are there new approaches to drug development that can provide more accurate information at a lower cost?

Greater predictability of efficacy and toxicity in humans before drugs move into clinical trials would lower the failure rate of new medications during clinical trials. One area of particular importance is differences between animal studies and clinical studies carried out in humans. Differing conclusions drawn between preclinical animal models and human trials usually appear during late-stage clinical or postmarket failures, when much time and effort has already been invested in the development of the drug. An example of discrepancies between species impacting the development pathway and timelines is found in the cholesterol-lowering statins.[5] Sankyo, a Japanese pharmaceutical company, developed the very first statin, Compactin. Initial tests of Compactin in rats did not lower cholesterol. Not until further tests were carried out in rabbits, monkeys, and dogs were the cholesterol lowering effects of Compactin observed. Compactin was initially successful at lowering cholesterol in human trials, but was withdrawn, due to suspected high-dose toxicity in dogs. Years later, after overcoming toxicity concerns in preclinical models, including a phase IV study in over 8,000 patients, Merck's lovastatin was approved by the FDA. Many adverse effects seen in the original animal studies were not detected in the clinic. Importantly, muscle damage in humans, a serious side effect, was not detected in the animal studies.[5] Hence, preventing these problems in the first place would improve the cost and time of bringing a drug to market. Are there tools available to address these issues?

To better predict problems with drug candidates in clinical trials, several existing and emerging technologies have been employed:

- Traditional two-dimensional (2D) tissue culture
- Conventional whole rodent models
- Humanized mouse models
- Three-dimensional (3D) culture models
- Co-culture systems
- 3D tissue models

Two-dimensional culture systems employ cell lines; the cells themselves contain numerous genetic mutations and lack important characteristics found in the tissues from which they were derived.[6] Traditional culture performed with primary cells offers characteristics more similar to their organs of origin.[7] But the shortcomings of this approach include obtaining enough cells for large-scale screens and the rapid loss of important protein and gene expression profiles usually within 48 hours.

To capture more of the tissue complexity and whole-body impact, animal studies have been the mainstay of toxicology. However, differences in basic physiology between the species lead to incorrect conclusions about a drug candidate's toxicity.

To improve upon the conventional 2D cell-based assays and animal studies used in drug development, more complex *in vitro* cell-based models, such as 3D culture models, co-culture systems, 3D tissue models, and humanized rodent models, are emerging. Some of these new technologies enable testing of drug effects in human systems (vs. animal models), eliminating the species differences that hamper interpretation of the outcomes. Moreover, by building assays that incorporate multiple human cell types and configuring the cells into 3D structures that mimic native tissue architecture, questions of safety and efficacy can be answered in a more *in vivo*-like context than traditional 2D cell cultures.

One such approach to testing human tissue-level effects of drugs or other treatments in a whole-animal context is humanized mice. Models include mice bearing human-derived tumors (xenografts) or mice in which the endogenous liver has been compromised and repopulated with human

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Table 1. Comparison of Key Features of *In Vitro* 3D Models

	Spheroids	*Cell-seeded scaffolds*	*Bioprinted tissue*
Tissue-like cellular density *in vitro*	✓	Limited	✓
True 3D; >250 μm in *x, y,* and *z* axes	✓	✓	✓
Multiple tissue-specific cell types	✓	✓	✓
Spatially controlled cell compartments	×	Limited	✓

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hepatocytes.[8] Xenografts are proving useful in oncology drug development, often enabling assessment of efficacy in the context of patient tumor phenotype and heterogeneity. Likewise, mice with humanized livers offer the ability to assess drug metabolism preclinically in the context of a human liver. With all humanized models, it is important to recognize that their chimeric nature—they are a single human tissue or cell type embedded within an otherwise murine system—may still cloud interpretation of outcomes due to species differences. For example, it has been shown that within a few serial passages, the stroma and vascular components of xenograft models are largely mouse in origin,[9] and while mice with humanized livers bear human hepatocytes, the other cell types found in the liver and all of the interrelated organ systems are mouse.[10] Humanized mice are not fully human; hence, they fall short in modeling human systems.

3D culture and co-culture systems have existed for multiple years; recent refinement has increased their availability for pharmaceutical research. Most systems can utilize human cells, and are composed of two to three primary cell types. However, the density of cells in 3D culture and co-culture systems is much less than that found in the *in vivo* environment. Some systems utilize scaffolds or artificial matrices to hold the cells together. Matrices and scaffolds introduce noncellular, artificial components into the model. These foreign factors add another nonhuman or nonnative aspect to the system—what effect will this scaffold have on the drug compounds it is used to test?

A more recent approach to generate 3D culture systems involves bioprinting. 3D bioprinting, for the purpose of this article, is defined as the automated fabrication of structures composed of living cells to form 3D ''tissues'' possessing critical attributes of the target tissue they represent. Some bioprinting approaches rely on the controlled deposition of polymeric materials, which may contain some proportion of cells. Other bioprinting methods leverage unique instrument platforms that enable 3D structures to be created from cells without dependence on polymeric materials, resulting in the formation of 3D tissues that are composed solely of human cells and the extracellular matrix they produce. 3D bioprinted tissues can be configured to mimic the cellular density of a target tissue, with consideration for cellular, matrix, and void space components. A summary of key features of these 3D models can be found in Table 1. Spatially defined deposition of two or more cell types enables the design and fabrication of tissues *in vitro* that mimic key aspects of the composition and architecture of the target tissue.

Scientific studies have demonstrated the benefits of spatial patterning in co-culture systems.[11,12] Since no tissue in the body is composed of a single cell type, the beneficial effects of including two or more cell types in an *in vitro* culture system are also predictable.[13] Additional studies have elegantly

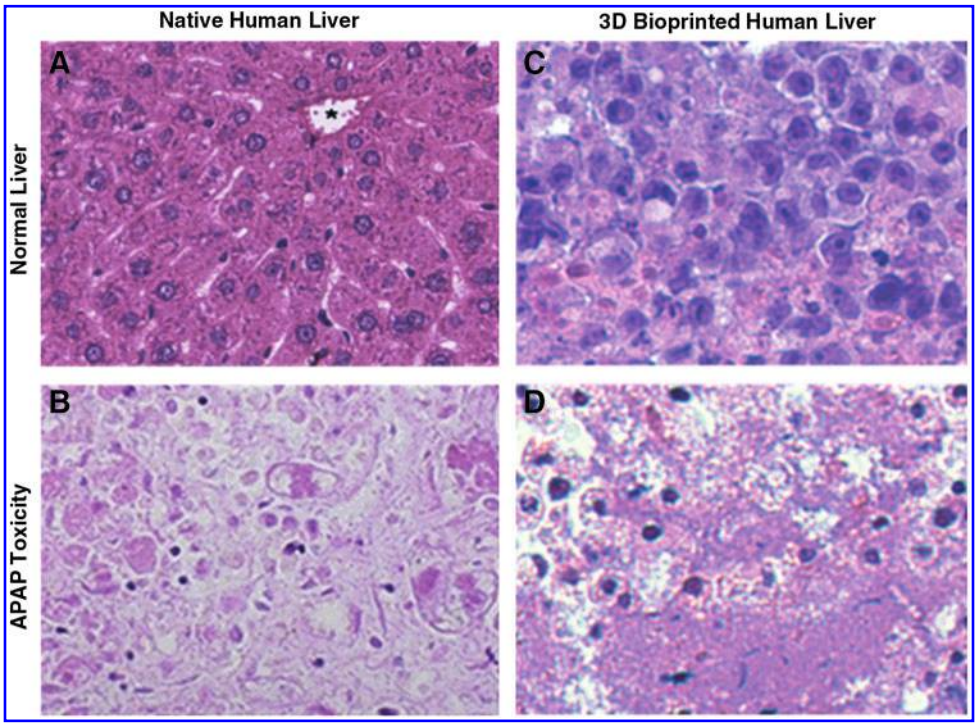

FIG. 1. Hematoxylin and eosin-stained human liver tissue and bioprinted human liver tissue. Panel **(A)** shows normal human liver, while panel **(B)** shows a severely damaged human liver from a patient with acetaminophen (APAP) overdose. Panels **(C)** and **(D)** are of bioprinted human liver tissue dosed with vehicle **(C)** or high dose APAP **(D)**. Regions of the bioprinted tissues display tissue damage similar to the clinical specimen shown in **(B)**. Note also that the density of cells in the bioprinted tissue is similar to the cell density of the whole organ. Used with permission of Organovo.

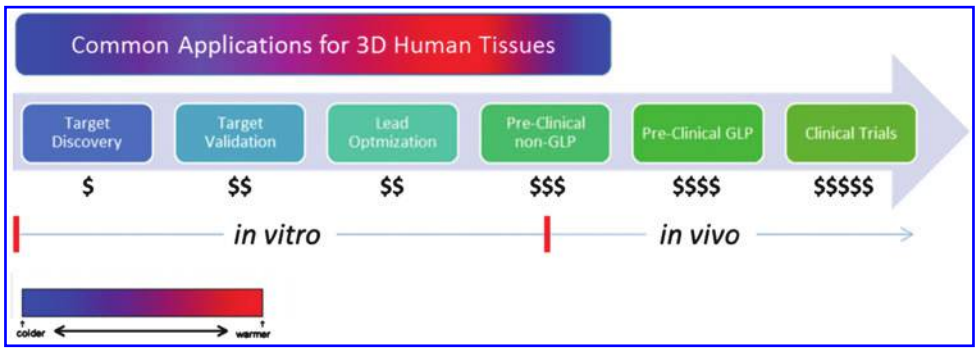

FIG. 2. Where does 3D tissue printing fit into drug discovery and development? Used with permission of Organovo.

demonstrated the benefits of spatial patterning in co-culture systems, wherein supporting mouse fibroblasts extend the lifespan and support functionality in islands of human hepatocytes.[14] With the recent innovations in bioprinting, it is now possible to combine the critical elements of three-dimensionality and spatial patterning to generate 3D *in vitro* tissue systems that recapitulate key architectural and functional features of human tissue *in vivo*.

In vitro applications for bioprinted human tissues include toxicity testing, metabolism, disease modeling, and efficacy testing. Moreover, with the advances in induced pluripotent stem cells (iPS) technology, it is possible to contemplate population-based studies of drug effects and personalized medicine strategies that employ bioprinted tissues as new-generation surrogates for clinical outcomes.

Organovo® is a San Diego-based biotech company considered to be an industry leader in the fabrication of 3D bioprinted human tissues. Created using a proprietary 3D bioprinting process, Organovo's bioprinted tissues are generated from primary human cells without dependence on integrated biomaterials or scaffolding to achieve three-dimensionality. Bioprinted tissues recapitulate relevant aspects of *in vivo* biology, including intercellular interactions and deposition of native tissue extracellular matrix. As a consequence, the tissues remain viable for extended periods of time *in vitro*, allowing the examination of chronic, low-dose drug treatments, and possess architectural and functional features that mimic crucial aspects of the native tissue environment. Biochemical, genomic, proteomic, and unique histologic endpoints can be assessed over time in drug discovery research or as a component of preclinical safety and efficacy testing programs. Organovo's exVive3D™ bioprinted human tissues may ultimately lower the risks and costs of drug development by enabling human tissue-specific data to be captured prior to initiating clinical trials.

Beyond toxicity, the potential to build human tissue models to study the development and progression of disease provides an enormous opportunity to enhance drug discovery and development by reducing cost and time. Cancerous bioprinted tissue models could be employed to study the primary and secondary effects of potential drug candidates. Advances in creating iPS allow samples from a specific patient to be dedifferentiated and then redifferentiated into tissues of choice. In the future, patient-specific tumor-derived cells could yield a personalized cancer ''tumor'' via bioprinted tissue models.

Liver is a particularly interesting tissue model to pursue; the liver metabolizes many drugs and is a common target. Organovo's exVive3D liver is a bioprinted human tissue model comprising primary hepatocytes, hepatic stellate cells, and endothelial cells. Histological examination of the model confirms intercellular junctions between the hepatocytes, microvascular structures composed of CD31-positive endothelial cells, and the presence of spatially defined compartments enriched for specific cell types. Tissue sections shown in Figure 1 demonstrate the structural similarities between slices of native liver and bioprinted liver tissue, with exhibition of damage caused by acetaminophen overdose.

Organovo's exVive3D liver tissue secretes albumin, fibrinogen, and transferrin proportional to levels in whole liver. Levels of ATP and lactate dehydrogenase are in the normal range for the 3D model as well. Toxicology studies also demonstrate an *in vivo*–like response to acetaminophen, acetaminophen, and ethanol, and diclofenac, a known liver toxin.

So what are the pragmatic implications for drug discovery and development with this new technology? 3D bioprinted human tissues have a huge potential to fill the gaps between lead optimization, preclinical non-GLP, and drug discovery phases. Additionally, 3D tissues hold promise in reducing the risk and cost associated with the later stages of drug discovery and development. These models offer a more accurate and reproducible tool for answering questions related to human biology at the tissue level. Presently, 3D bioprinted human tissue models cannot replace traditional, 3D culture, co-culture, or animal models. However, a better predictive tool in the toolbox is necessary. Potential times for the application of 3D bioprinted tissues can be seen in Figure 2. The ability of drug discovery and development researchers to make decisions with a higher level of confidence at an earlier stage can only be seen as a positive outcome in this high-cost, high-risk game.

Author Disclosure Statement

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