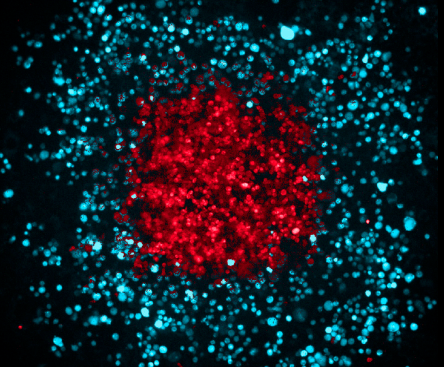

Biopixlar® Application Note

Bioprinted composite in vitro liver model

INTRODUCTION

The discovery of novel and effective drugs is hampered by a low likelihood of acceptance (LOA), where more than 90% of all clinical drug candidates fail.[1-2] The selection of drug candidates is based on in vitro and in vivo experiments that largely fail to recapitulate the bona fide physiological effects in humans, calling for better predictive human surrogate models for drug discovery.

An important step in providing an alternative to 2D culture and animal models is the advancement of 3D models, where different cell types are combined in a 3D environment to mimic a tissues' extracellular matrix, biochemical and mechanical cues as well as cell: cell interactions seen in vivo.[3] More importantly, producing and combining multiple human tissue surrogate models that together demonstrate a biological system, eventually replacing animal models, is in line with the 3R principle (Refine, Reduce and Replace the use of animals).

The non-alcoholic fatty liver disease (NAFLD) is a chronic disease that affects 20-50% of the population[4], recognized by an abnormal accumulation of fat, which may eventually lead to liver failure and the need for liver transplantation[5]. Current models to target NAFLD include liver spheroids made by using ultralow attachment plates and the seeding of cells[6,7], applicable for high-throughput screening. However, the models are simple in nature by the lack of in vivo cell, microenvironment and structural heterogeneity of the liver[8].

Here, we take advantage of the Biopixlar bioprinting platform (Fluicell) to carefully position hepatocyte and fibroblast cells in suspension onto a feeder layer of fibroblast cells. The 3D liver-model functionality was measured by the amount of secreted albumin, where it showed a significant increase of albumin production compared to a mono- or co-culture of cells printed as a single cell layer. Thus, the ability to print confluent patches of cells onto cells with direct cell-cell interaction will influence tissue model function and thus have an importance in the future development of in vivo like model systems.

BIOPIXLAR PRECISION BIOPRINTING

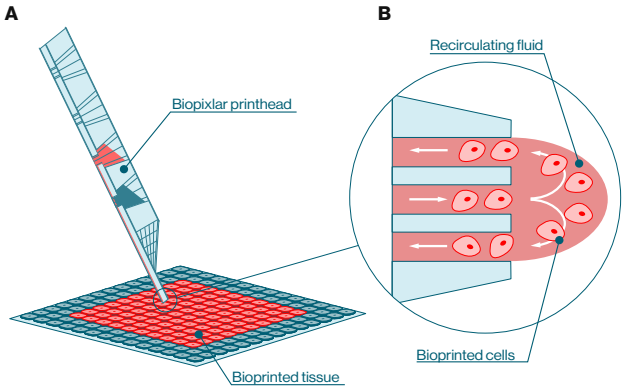

Figure 1. A) Schematic illustration of the Biopixlar printhead loaded with two different cell types, generating a composite tissue through direct cell printing. **B)** Drirect cell printing is enabled by a recirculating flow that restricts the cell ejection to a precisely controlled volume.

The detailed cell composition of the composite liver model is enabled by the microfluidic Biopixlar bioprinting technology. To enable precise cell placement, the Biopixlar system uses a microfluidic recirculating flow (Figure 1) to print cells directly in culture media without any support material.[9]

The bioprinting system uses an exchangeable microfluidic printhead with the capacity to contain up to four different cell types or solutions simultaneously to enable rapid assembly of detailed in vitro models.

Intercellular interaction — Microfluidic recirculating flow deposition enables precise placement of cells and allows creation of detailed features that promote intercellular communication.

Biological relevance — Complex tissue models can be constructed with improved physiological response compared to conventional cell cultures.

Viability — Printing occurs directly in culture media with minimal mechanical stress on the cells enabling high cell viability.

LIVER MODEL PRINTING

1 Create supporting fibroblast layer

The liver model is constructed by first assembling a support layer of fibroblast cells (3T3-J2), deposited in a circular shape of approximately 2 mm in diameter. To create a confluent cell layer, the supporting fibroblast cells are incubated for 24 hours

2 Print hepatocyte structure

Prior to printing cells onto the supporting layer of fibroblast cells, a cell-adhesion agent is printed onto the fibroblast cells in a desired shape. This allows for cell attachment during the printing of the second layer of cells.

Hepatocyte cells (HepG2) are then printed onto the supporting cell layer in a 0.5 x 0.5 mm2 square. During printing, cells are ejected from the microfluidic printhead in a controlled fashion using manual or automated stage positioning.

3 Print surrounding fibroblast layer

Following the printing of hepatocyte cells, a layer of fibroblast cells is printed around the square of hepatocyte cells, forming a square top cell layer with a total area of 1 x 1 mm^2. The fibroblast cells will function to promote the metabolic capacity of the hepatocytes

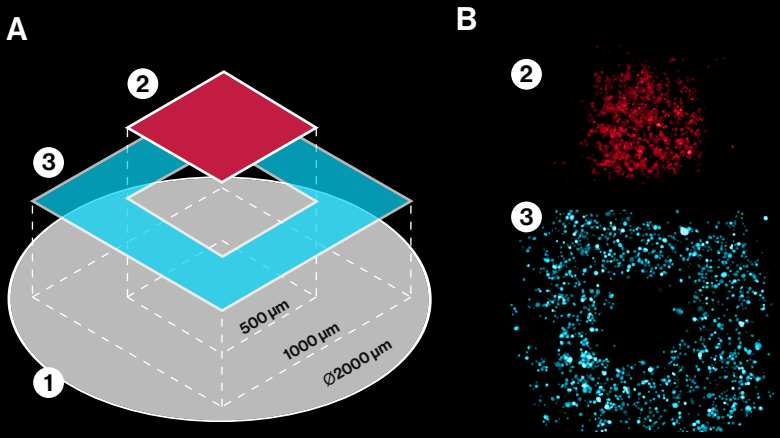

Figure 2. A) Three stage liver model assembly showing fibroblast support layer (1), hepatocyte structure (2) and surrounding fibroblast layer (3). **B)** Fluorescent images of hepatocyte layer (2, red) and surrounding fibroblast layer (3, blue) directly after printing.

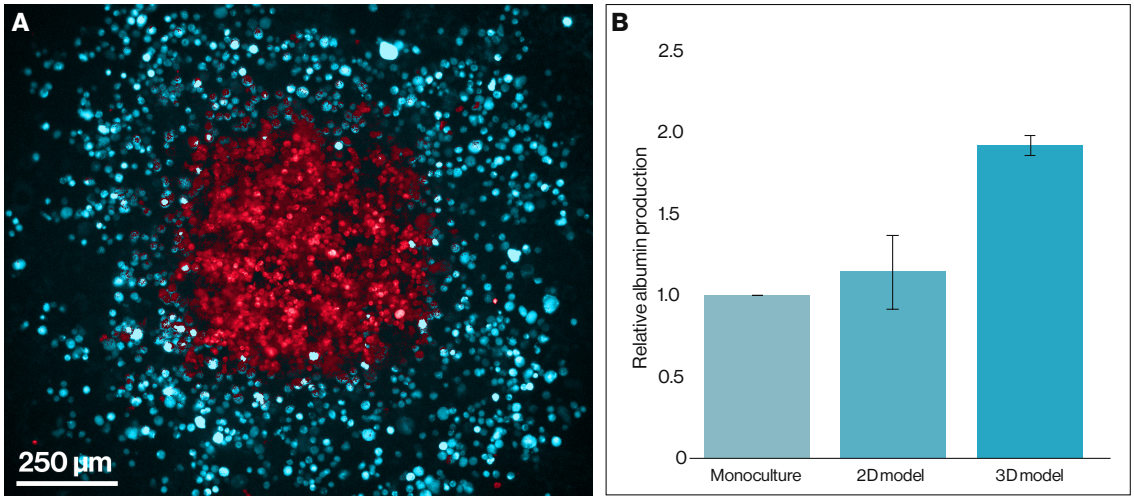

Figure 3. A) Fluorescence microscopy image of the full top cell layer with hepatocytes (HepG2, red) surrounded by fibroblasts (3T3-J2, blue) **B)** Comparison of albumin production in hepatocyte monoculture, 2D coculture and the 3D model presented here. Albumin measured after 7 days incubation.

LIVER MODEL FUNCTIONALITY

The functionality of the in vitro liver model was evaluated by measuring albumin production after 7 days of incubation. Albumin production is one of the key functions of hepatocytes and is recognized as an important biomarker for hepatocyte functionality. Conventional 2D cell cultures display a gradually decreasing albumin production over time, which is indicative of decreasing hepatocyte functionality.[10] Having a cell-based model system that can better maintain albumin production is therefore desirable.

To assess the model functionality, we compared the 3D bioprinted composite model with both a conventional hepatocyte culture and a 2D composite model. The results presented in Figure 3 show a significant increase in albumin production for the 3D Biopixlar model after 7 days incubation, compared to the 2D composite and monoculture systems.

CONCLUSION AND DISCUSSION

Fluicell's microfluidic based bioprinter offers a novel way to create advanced in vivo-like tissue models for functional assays or drug toxicity screening. The results presented here show that liver models created using the single-cell printing capacity of Biopixlar have greatly improved hepatocyte albumin production compared to the monoculture system, resulting in increased biological relevance. Furthermore, the model presented here can easily be expanded upon by adding additional hepatocyte structures, increasing the cell pattern complexity or by adding additional cell types.

The high bioprinting precision and cell viability enables creation of functional cell-based research models with a high degree of biological relevance, making Biopixlar a powerful tool for biomedical research and drug discovery.

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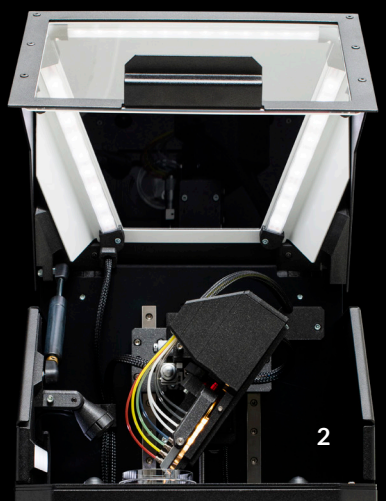
ABOUT BIOPIXLAR®

Biopixlar is Fluicell's family of high precision 3D bioprinting platforms. The Biopixlar platforms uses Fluicell's innovative open volume microfluidic technology and is capable of creating tissues, 3D cell cultures and cell arrays with single-cell precision. Biopixlar desposits cells directly in solution without any bioink, which ensures high cell viability and efficient intercellular communication. Biopixlar is available in two verions: as the modular Biopixlar platform and as the more compact Biopixlar AER.

ABOUT FLUICELL®

Fluicell is a Swedish life science company, specializing in high precsion research tools for biological and pharmaceutical research, in vitro disease models and cell-based regenerative medicine research and development. Fluicell provides innovative research instruments for single-cell biology and 3D bioprinting, based on proprietary microfluidic technology.



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