

Creating a 3D multicellular liver model using Biopixlar single-cell bioprinting

Biopixlar single-cell bioprinting

Biopixlar is a completely new bioprinting system based on Fluicell's innovative microfluidic technology. With Biopixlar, you can create multicellular tissues with single-cell precision directly in culture media. Biopixlar allows you to deposit cells right where you want them, without using any bioink.

Print only the cells that you want!

- **Biological relevance** – Complex tissue models can be constructed with improved physiological response compared to monoculture systems.
- **Simplicity** – Users have direct control of cell placement through Fluicell's easy-to-use interface.
- **Viability** – Printing occurs directly in culture media with minimal mechanical stress on the cells enabling >95% cell viability.

Liver model construction

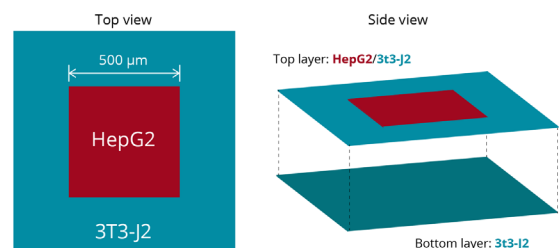
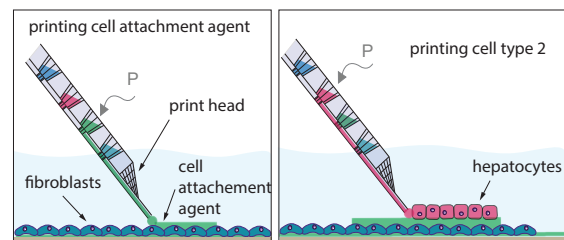
The multicellular in vitro liver model is created by assembling two separate cell layers on top of each other. The two layers are held together with a connecting cell-adhesive layer.

Bottom layer

The bottom layer consists of a larger uniform patch of 3T3-J2 fibroblasts acting as an underlying support structure.

Top layer

The second layer is the functional part of the model, built from a central patch of hepatocytes (HepG2) with a surrounding fibroblast structure.



Liver model printing

Step 1: Print supporting fibroblast layer

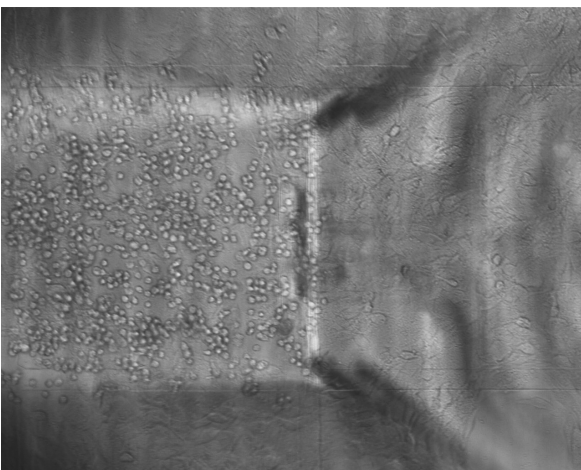
The liver model is constructed by first assembling a support layer consisting of fibroblasts (3T3-J2). To prepare for the printing of the hepatocyte layer, cell-adhesive agent is printed on top the fibroblasts. This allows adhesion of the second layer of cells.

Step 2: Print hepatocyte structure

Hepatocytes (HepG2) are printed on top of the support layer in a 0.5 x 0.5 mm square. During printing, cells are ejected from the microfluidic printhead in a controlled fashion. The desired pattern is obtained by moving the substrate while cells are being ejected.

Step 3: Print surrounding fibroblast layer

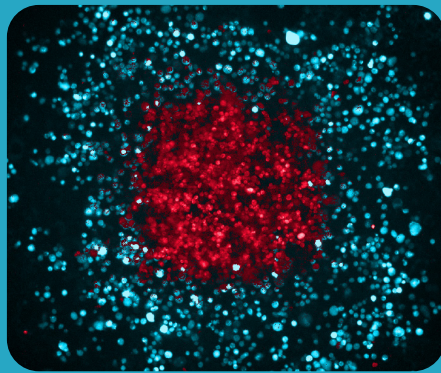
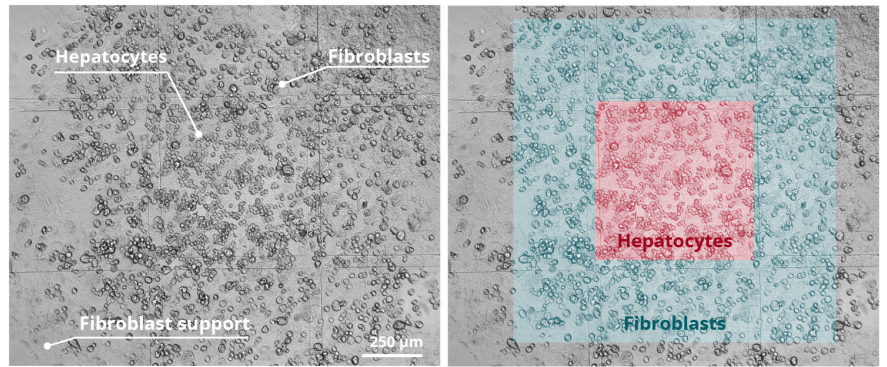
Following this, a layer of fibroblast is printed around the hepatocyte square, shown in detail in the second video. The fibroblasts help to promote the metabolic capacity of the hepatocytes.



Liver model composition

The image to the right shows the complete liver model with hepatocytes printed on top of and surrounded by a layer of fibroblasts. The colored regions in the right panel highlight the structure of the top layer of cells, with hepatocytes in red and fibroblasts in blue.

Creating detailed structures with complex architectures on the cellular level is both fast and simple with Biopixlar. Both printhead positioning and printing speed can be controlled directly by the user, providing a wide range of possibilities in model design.

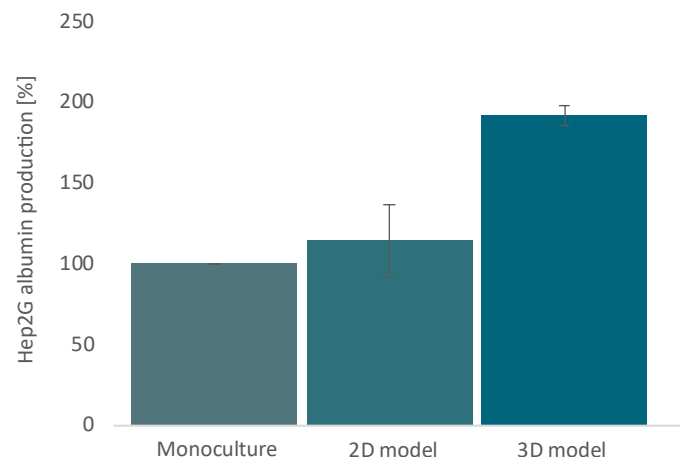


To facilitate distinction between the different cell types, the cells are fluorescently labeled. The image below shows the composition of the liver model directly after printing in bright field and fluorescence imaging. In the fluorescence image, the hepatocytes appear in red and the fibroblasts in blue.

Liver model functionality

Albumin production, which is one of the **important functions of hepatocytes**, was used to probe the functionality of the liver model created using Biopixlar. The results presented below show a **significant increase in albumin production** for the 3D Biopixlar model after **7 days incubation**, when comparing to our bioprinted 2D and monoculture tissues.

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, it is easy to further increase model complexity with Biopixlar, either by building additional cell structures or by adding additional cell types



Fluicell AB
Flöjelbergsgatan 8C
SE – 431 37 Mölndal, Sweden

www.fluicell.com
info@fluicell.com



AXT PTY LTD
Authorised Distributor
Fluicell
Australia & New Zealand

1/3 Vuko Place
Warriewood
NSW 2102 Australia

+61 (0)2 9450 1359
axt.com.au
info@axt.com.au

