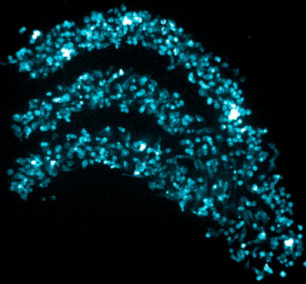

Biopixlar® Application Note

Microenvironment modeling with high precision cell and protein bioprinting

INTRODUCTION

In the field of pharmaceutical science, it is pivotal to combine high throughput with physiologically relevant models to readily produce reliable data for drug discovery. The high-throughput models are predominantly designed using mono- or co-cultures of cells seeded on coated or treated plastic 2D surfaces, whereas the conclusions of such models are verified in systemic model systems (e.g., animals).

There are several fundamental challenges to designing a model system that predicts the effect of drugs in humans, as a measurement of cell response. These include the ability to incorporate biochemical and mechanical cues, tissue architecture and endocrine signaling from a physiological microenvironment, as well as ethical reasoning for the use of animals.

As an important alternative method, in vitro 3D cell cultures have emerged and provide relatively advanced physiological models compared to 2D cell cultures.[1] In fact, the US congress has recently approved the FDA Modernization Act 2.0, allowing alternative methods to be used instead of animal testing for drug approval by the FDA[2], thereby opening for the possibility to replace animal models with 3D cell culture systems.

In efforts made to progress the development of 3D cell cultures, focus has been directed toward the ability to control material performance as well tissue model architecture, cell viability and cell function by using different bio- and 3D-printing technologies.[3]

Here, we present a a method to create detailed 3D cell cultures based on high precision microfluidic bioprinting.[4] The method is desiged to provide a high level of cell microenvironment control by combining bioprinting of sparse amounds of cells in suspension with minimal shear stress together with precisie depostion proteins and other molecules. The method produces confluent patches of cells with direct cell-cell and cell-microenvironment (non-carrier material) modeling with single-cell printing resolution. Thus, the printing methodology allows for precise microenvironment modeling, essential for building reliable cell and tissue models used in drug discovery or fundamental sciences.

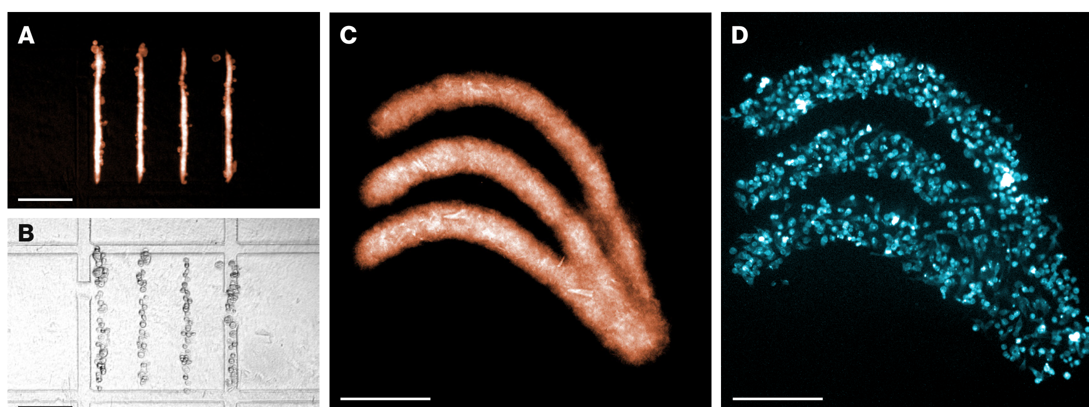

Figure 1. Cell attachment agent and cell printing. Cell attachment agent (Poly-L-Lysine conjugated FITC) was printed (A, C) prior to printing HaCaT (B) and HepG2 (D). Cells in panel (D) stained with CellTrace violette. Scale bar 200 µm. Fluorescence micrographs are false-colored.

RESULTS

The binding of the positively charged cell attachment agent (CAA) to the mesh-like avidinated propriety substrate was studied using a FITC conjugate. Here, using an automated printing mode of the Biopixlar platform, the CAA was shown to bind to the propriety material (Figure 1).

By adjusting the flow-zone using the software pressure settings, thin lines of CAA was successfully printed (Figure 1A). In a similar manner, the pressure settings were adjusted to make a larger flow-zone, resulting in wider lines being printed onto the substrate (Figure 1C). Following the printing of the CAA-FITC conjugate, cells were printed and shown to bind specifically to the CAA pattern (Figure 1B and 1D). Thus, the precise pattering of the CAA allows for the making of cell models with a precise architecture at a single cell resolution.

We further advanced the model design by printing a biotinylated molecule to the avidinated surface prior to printing CAA and cells, using the well-known avidin-biotin interaction as a generic mean to bind molecules to the surface, which principle allows for the usage of any available biotin-coupled molecule (commercially or in-house) (Figure 2A). Here, a fluorescent biotin-atto590 conjugate was printed in an “F” shape, followed by CAA and cells (Figure 2B). The biotin molecule was shown to bind to the substrate and remain on the substrate post printing of CAA and cells, as measured by fluorescence (Figure 2C).

Following the printing of cells, Cholesterol-PEG-Biotin and an Avidin-FITC conjugate were printed sequentially in a circular pattern onto the cells. In contrast to the mesh-like fluorescence given by the binding of biotin-atto590 to the substrate, the Avidin-FITC molecule was shown to bind to the cell membranes of the cells pre-printed with cholesterol-PEG-biotin (Figure 2C), as measured by fluorescence.

The model of printing molecules and cells onto a substate was then applied for printing cell layers. The ECM protein fibronectin, conjugated with biotin, was printed in an “F” shape onto the mesh-like substrate, followed by CAA and cells stained with CellTracker Red (Figure 2D). Similarly, cells stained with CellTrace Violette were printed as a dot (Figure 3E). Following the printing of the first layer of cells, cholesterol-PEG-biotin + avidin + fibronectin-biotin + CAA was sequentially printed onto the “F” shaped cell model, followed by a second layer of cells stained with CellTrace Violette. The model was imaged with fluorescence and phase holographic microscopy (Figure 3F).

CONCLUSION AND DISCUSSION

The ability for cells to interact with the microenvironment is essential when designing physiologically relevant tissue models. By using the Biopixlar bioprinting platform by Fluicell, molecules were successfully printed onto substrates and cells, using an approach taking advantage of the high affinity binding between avidin and biotin, thus generating a generic system for molecule printing. Equally important, this methodology was applicable for printing cells on cells and thus represents a powerful tool in creating heterogenic microenvironments. In addition, this bioprinting technology gives the ability to control cell confluency in a repetitive and precise manner, here illustrated by printing cells as an “F” in two layers.

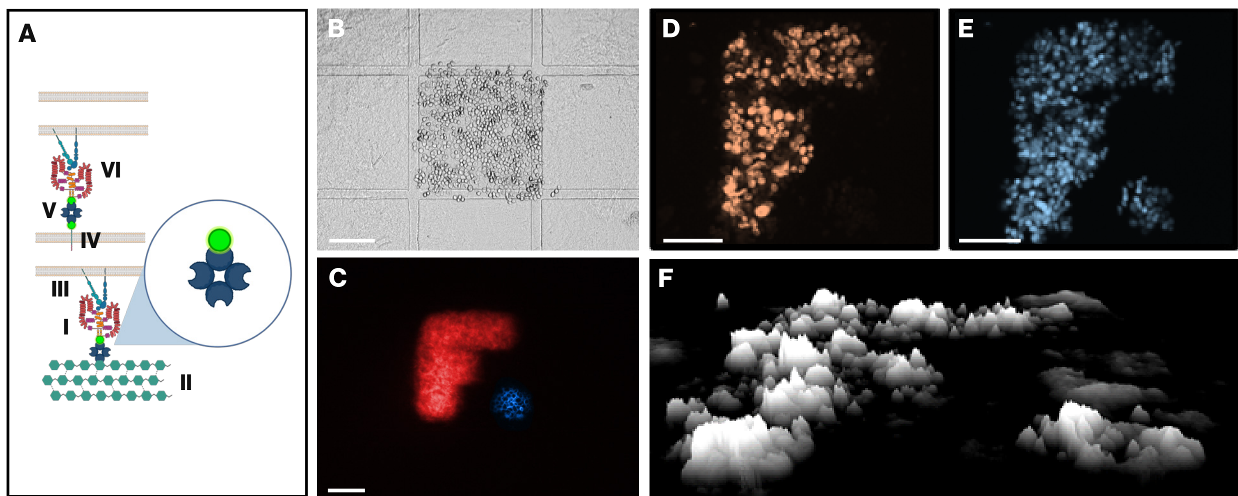

Figure 2. (A) Schematic image of a biotinylated molecule (I) anchored to an avidinated substrate (II), cell binding to the bioprinted molecule (III), and a cholesterol-PEG-biotin (IV) + avidin (V) + biotinylated-fibronectin molecule (VI) sandwich residing on the cell. The dual bilayers in the illustration indicate the two different cell layers (HaCaT). (B, C) Biotin-Atto was printed in an "F" shape onto a mesh-like proprietary material, followed the sequential printing of poly-L-Lysine containing a trace of fluorescein and cells. (C) Following the printing of cells, Cholesterol-PEG-biotin and Avidin-FITC were sequentially printed as a circular shape onto the cells. (D) Printing molecules prior and post printing of the first layer of cells. Biotin-fibronectin was printed onto an avidinylated substrate, followed by the sequential printing of CAA containing a trace of fluorescein and cells stained with CellTracker red to make a "F" shaped cell model. (E) As in (D), cells stained with CellTrace Violette were printed as a dot. The "F" shaped layer of cells was sequentially printed with Cholesterol-PEG-biotin + Avidin + fibronectin-biotin + CAA containing a trace of fluorescein, followed by CellTrace Violette stained cells. (F) The printed cell layers were imaged using phase holographic microscopy. Scale bar 200 µm. Fluorescence micrographs are false-colored.

METHODOLOGY

Cell culture

HEPG2 (Sigma) and HaCaT (AddexBio) cells were maintained according to producers' recommendation and cultured in cell culture treated flasks (Falcon).

Surface

A flat proprietary mesh-like substrate was prepared according to internal standard protocols.

Cell preparation

A sub confluent flask of cells was washed in PBS (Cytiva), detached using Accutase (Gibco), washed in media by centrifuging for 3 min at 300 G and resuspended in PBS. Following the resuspension of cells, HepG2 cells were syringed 3× (total volume) using a 25 G needle (BD) and filtered using a 40 µm cell strainer (Fisher scientific).

Single cell suspensions were washed once in PBS by centrifugation at 300 G for 3 min and counted. For cell printing without staining, one million cells were aliquoted, centrifuged at 300 G for 3 min and resuspended in PBS with a final concentration of 15 mg/ml of PEG6K (Fisher Scientific) at 10^6 cells/ml in a total volume of 100 µl.

For staining, one million cells were aliquoted, centrifuged at 300 G for 3 min, resuspended in a total volume of 1 ml with 5 µM CellTracker red CMTPX (Invitrogen) or 5 µM CellTrace Violette (Invitrogen) in PBS (Cytiva), incubated for 5 min at room temperature, washed once in PBS (Cytiva) by centrifugating at 300 G for 3 min and resuspended in PBS with a final concentration of 15 mg/ml of PEG (Fisher Scientific) at 10^6 cells/ml in a total volume of 100 µl.

Molecules

The following molecules were used: Biotin-Atto590 (Merck) at 10 ug/ml in PBS (Cytiva); 0.5 mg/ml poly-L-lysine (Sigma) with a trace of 200 µM fluorescein (Fisher) in PBS (Cytiva); 50 µM of Cholesterol-PEG-Biotin of 2 kDa (Nanocs) in PBS (Cytiva); 2 mg/ml of Avidin-FITC (Fisher Scientific) in PBS; 1 mg/ml Fibronectin-Biotin (Cytoskeleton) in PBS (Cytiva); 10 mg/ml Avidin (Fisher scientific) in PBS (Cytiva).

Printheads

Printheads of 30 µm and 50 µm were prepared according to manufacturer's recommendations (Fluicell) and used for molecules or cells respectively.

Cell and molecule printing

The printing of molecules was executed using an automated printing protocol made with the Biopixlar software (Fluicell), whilst cells were printed in manual mode using the Biopixlar associated gamepad (Fluicell), at standard pressure settings, fine-tuned to make a flow zone of 50 µm. Non-fluorescent conjugated molecules were printed with a trace of fluorescein to track the flow-zone size.

Analysis

Images were taken using the built-in bright field and fluorescent microscopy of the Biopixlar bioprinting platform (Fluicell) and phase holographic microscopy (PHI).

REFERENCES

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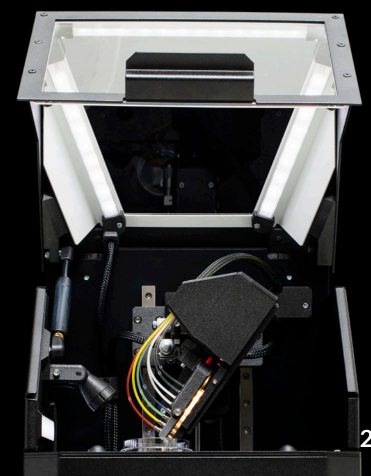
ABOUT BIOPIXLAR®

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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