Biopixlar® Application Note

Human de-cellularized tumors as bioprinting scaffolds

INTRODUCTION

Cancer is the leading cause of death of people less than 65 years of age, where the main diagnosed forms of cancer per 2023 was breast, lung, and prostate cancer.[1] Several studies have shown the importance of the tumor microenvironment on tumor preogression and patient outcome[2,3], underscoring the necessity to include the tumor microenvironment in cancer models. Specifically, this includes mechanical properties, biochemical cues, and inhabiting cells.

Consequently, studies using biomaterials to produce cancer models focus on mimicking these properties whilst maintaining biocompatibility.[4,5]

However, the complexity of the heterogenic tumor microenvironment makes it challenging to produce bonafide tumor models.

Here, Fluicell's microfluidic bioprinter is used to position cells directly onto de-cellularized and sectioned tumors without the use of additional biomaterials to produce advanced co-culture models. This novel approach of producing tumor models unlocks the possibility for complex studies on cell: tumor microenvironment and cell: immune cell interactions, cancer cell invasion, and drug toxicity.

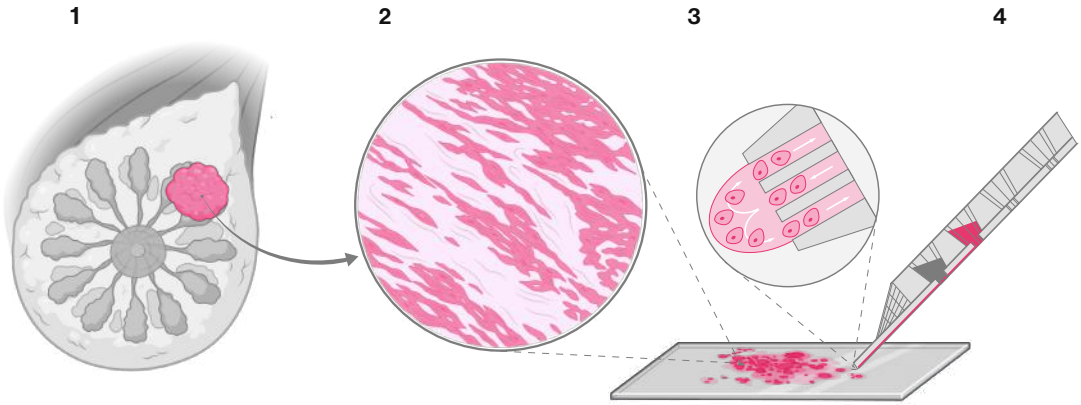

Figure 1. Workflow. Human derived breast tumors are collected (**1**), de-cellularized and frozen sectioned (**2**), and used as a substrate for microfluidic cell printing (**3**) using a printhead of Fluicell's bioprinting platform Biopixlar (**4**). Image created using BioRender.com.

RESULTS

De-cellularized and sectioned tissues were shown to have a heterogenic structure (Figure 2A) and and to be suitable for micrometer precision bioprinting by the sections' flatness and ability to bind Poly-L-lysin, a cell attachment agent. Adipose cells (Figure 2B) were successfully printed onto, and propagated in, the tumor section (Figure 2C). After 4 days, the triple negative breast cancer (TNBC) cells MDA-MB-231 (Figure 2D) were successfully printed onto the site of previously printed adipose cells (Figure 2E).

Following printing of the TNBC cells, the tissue was matured for 3 days and stained with FDA and Lipid Spot (Figure 3A), showing the presence of lipids in a densely packed region of cells. The mature tissue was treated with 4 µM doxorubicin for 72h and stained with FDA/PI in control (Figure 3B) and treated sample (Figure 3C) to study drug toxicity. Consistent with previous studies[6], a large portion of cells stained dead at 4 µM doxorubicin. However, there were still viable cells with an elongated phenotype, indicating the presence of drug resistant cells.

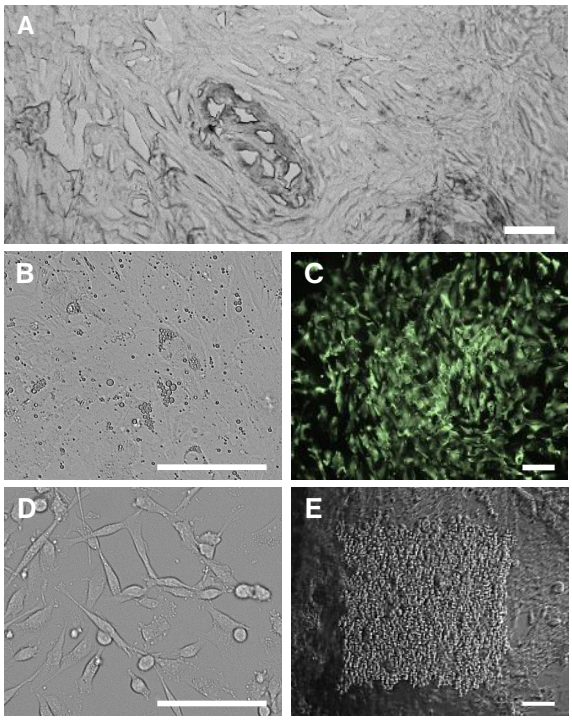

Figure 2. Tissue model preparation. **(A)** De-cellularized tissue section, **(B)** adipose and **(D)** TNBC cells were used to build a breast cancer model by printing **(C)** adipose cells followed by **(E)** TNCB cells onto the section, separated by 7 days of culture. Cells in were stained with FDA. Scale-bar 100 µm.

CONCLUSION AND DISCUSSION

The microenvironment influence tumor development by affecting the behavior of inhabiting cells and is important to consider when designing in vitro tumor assays. Here, we have used sectioned and de-cellularized patient-derived breast cancer tumors as a substrate for cell printing. The plane sections were suitable for micrometer positioning of the bioprinter printhead as well as binding the cell attachment agent and cells using Fluicell's microfluidic bioprinting platform. Importantly, the co-culture of cells propagated in the tissue sections to form highly dense cell patches seen in tissues. Thus, the tissue model represents a novel bioprinted and in-vivo like model for cancer drug toxicity, cancer cell invasion, and advanced co-cultures.

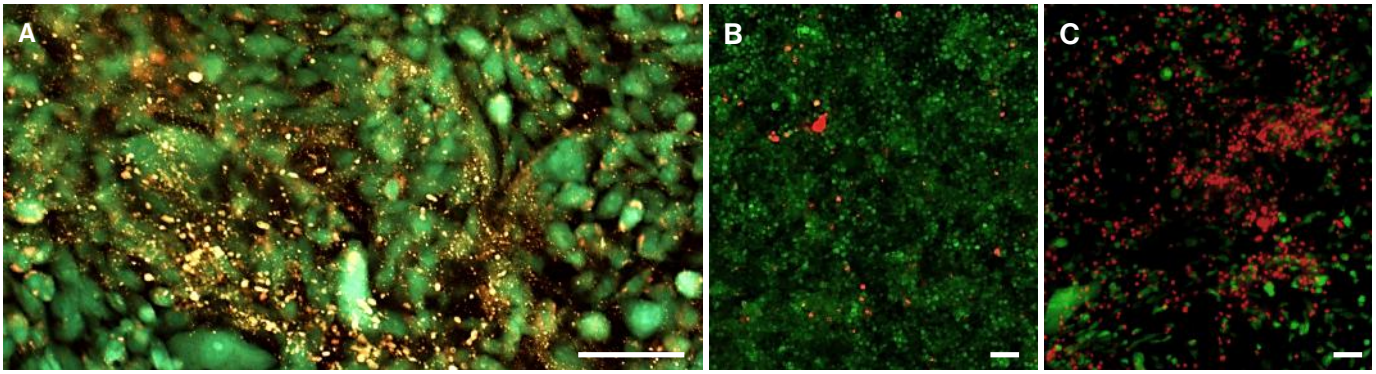

Figure 3. Drug toxicity. (A) The mature tissue was treated (B) with 4 µM Doxorubucin or (C) DMSO control for 72h and stained for live/dead cells. Panel A was stained with FDA (Green) and LipoSpot (Orange). Panel B and C were stained with FDA (Green) and PI (red). Scale-bar 100 µm.

METHODOLOGY

Tissue sectioning and de-cellularization

De-cellularized human breast tumors were prepared by Fidelis, a BioIVT company, using a previously published method.[3] Briefly, fresh tumors of 3–5mm were snap-frozen using liquid nitrogen and decellularized in lysis buffer (0.1 % SDS, 0.02 % sodium azide, 5 mM EDTA-$Na_2 \cdot 2H_2O$, 0.4 mM phenylmethylsulfonyl fluoride).

De-cellularized tumors were washed sequentially in lysis buffer without SDS, distilled water and PBS, followed by sterilization in PBS with 0.1 % peracetic acid. Sterilized de-cellularized tumors were washed in PBS with 1% antibiotic-antimycotic and stored in PBS containing 0.02 % sodium azide and 5 mM EDTA-$Na_2 \cdot 2H_2O$ at 4 °C.

Tumors were sectioned frozen (without OCT) as 5 µm, placed on an objective glass, heat-fixated at 45° C and shipped frozen in PBS containing 1 % antibiotic-antimycotic.

Cell culture

Bone marrow mesenchymal stem cells (BM-MSC) (Promocell) were cultured according to manufacturer's instructions in complete media (Promocell) supplemented with 1 % penicillin/streptomycin (Gibco).

At 80-90 % confluency, the BM-MSC were partially differentiated into adipose cells for 7 days prior to printing the cells on tissue sections by exchanging the complete media with differentiation media (Promocell). MDA-MB-231 cells were cultured according to manufacturer's instructions in RPMI 1640 (Gibco) supplemented with 10 % heat-inactivated FBS (Gibco) and 1 % penicillin/streptomycin (Gibco).

Prior to cell printing, BM-MSC and MDA-MB-231 cells were detached using StemPro Accutase (Gibco), washed in complete media, counted, centrifuged at 300 G for 5 min and resuspended in 15 mg/ml PEG 6000 (Hampton Research) in complete media.

Cell printing

De-cellularized breast tumor sections were washed sequentially in PBS (Cytiva) and ultrapure water (Invitrogen), dried at room temperature, and placed in an open chamber with BM:MSC differentiation media (Promocell).

A 50 µm printhead (Fluicell) was purged and prepared according to manufacturer's instructions, loaded with 0.5 µM Poly-L-lysine (PLL) (Sigma) containing a trace of 200 µM fluorescein (Fisher Brand), and printed onto the de-cellularized breast tumor sections at 30 µm/s using standard pressure settings.

Following printing, partially differentiated adipose cells at a concentration of 5×10^6 cells/ml were printed onto the previously printed site of PLL and incubated for 5 days at 5 % CO_2. Prior to printing MDA-MB-231 cells, the mature adipose cells were stained for 5 min with 8 µg/ml FDA (Fisher chemicals), washed 3 times with PBS (Cytiva) and placed in a co-culture medium consisting of 75 % differentiation medium (Promocell) and 25 % RMPI 1640 supplemented with 10 % heat-inactivated FBS (Gibco). The MDA-MB-231 cells were then printed directly onto the tissue section (without prior printing of PLL), previously printed with adipose cells, at a concentration of 10×10^6 cells/ml.

The tissue model was cultured 3 days prior to drug treatment. An endpoint sample was washed in PBS, sequentially stained with 8 µg/ml FDA (Fisher chemicals) and 1/2000 LipoSpot 488 (Biotium) for 10 and 30 min respectively in PBS, washed in PBS and imaged (Zeiss).

Doxorubicin treatment

Media was exchanged to co-culture media containing 4 µM Doxorubicin (Selleckchem) or DMSO (MP Biomedical) as a control and cultured for 72 h at 37° C and 5 % CO2. Cells were washed once in PBS (Cytiva), stained with 8 µg/ml FDA (Fisher chemicals) and 20 µg/ml PI (Sigma) in PBS (Cytiva) for 10 min at room temperature, washed 3 times in PBS (Cytiva) and imaged (Zeiss).

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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