Human de-cellularized tumors as bioprinting scaffolds

Cancer is the leading cause of death among people aged 65 years or less, where the main diagnosed forms of cancer per 2023 were breast, lung, and prostate cancer.[1] Several studies have shown the importance of the tumor microenvironment on tumor characteristics 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 cancer models. Here, Fluicell's microfluidic bioprinter is used to position cells directly onto de-cellularized and sectioned tumors without the use of synthetic biomaterials to produce advanced cancer co-culture models. This novel approach of making cancer models will allow the scientific community to, among others, advance studies on cell-tumor and cell-immune cell interactions, cancer cell invasion, and drug toxicity.

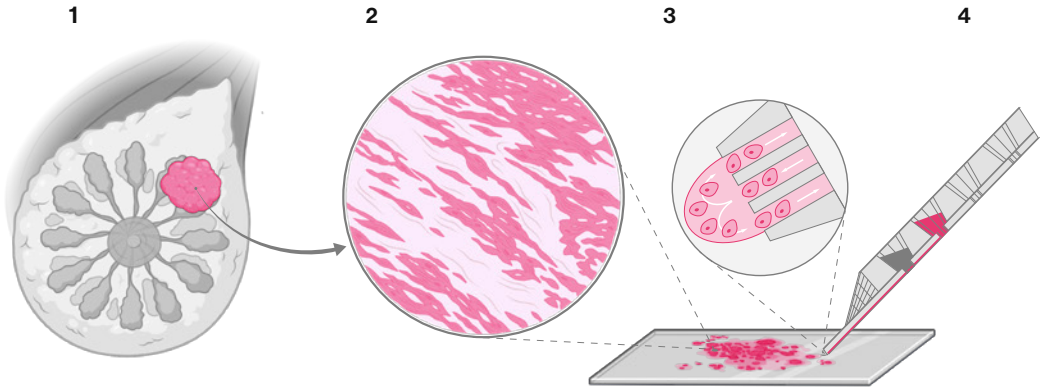

Figure 1. Workflow. Human-derived breast tumors are collected (1), de-cellularized and frozen sectioned (2), and used as a substrate for microfluidic cell printing (3) using a printhead of Fluicell's bioprinting platform Biopixlar (4). Image created using BioRender.com.

Results

De-cellularized and sectioned tissues were shown to have heterogenic structure (Figure 2A) and were suitable for micrometer precision printing by the sections' flatness and ability to bind a cell attachment agent (Poly-L-lysine). 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 printed on the same site as the 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 2F), showing the presence of lipids in a densely packed region with cells. The mature tissue was treated with 4 µM doxorubicin for 72h and stained with FDA/PI in control (Figure 2G) and treated sample (Figure 2H) to study drug toxicity. Consistent with previous studies6, a large portion of cells stained dead at 4 µM doxorubicin. However, there were still viable cells with an elongated phenotype, indicating drug resistant cells.

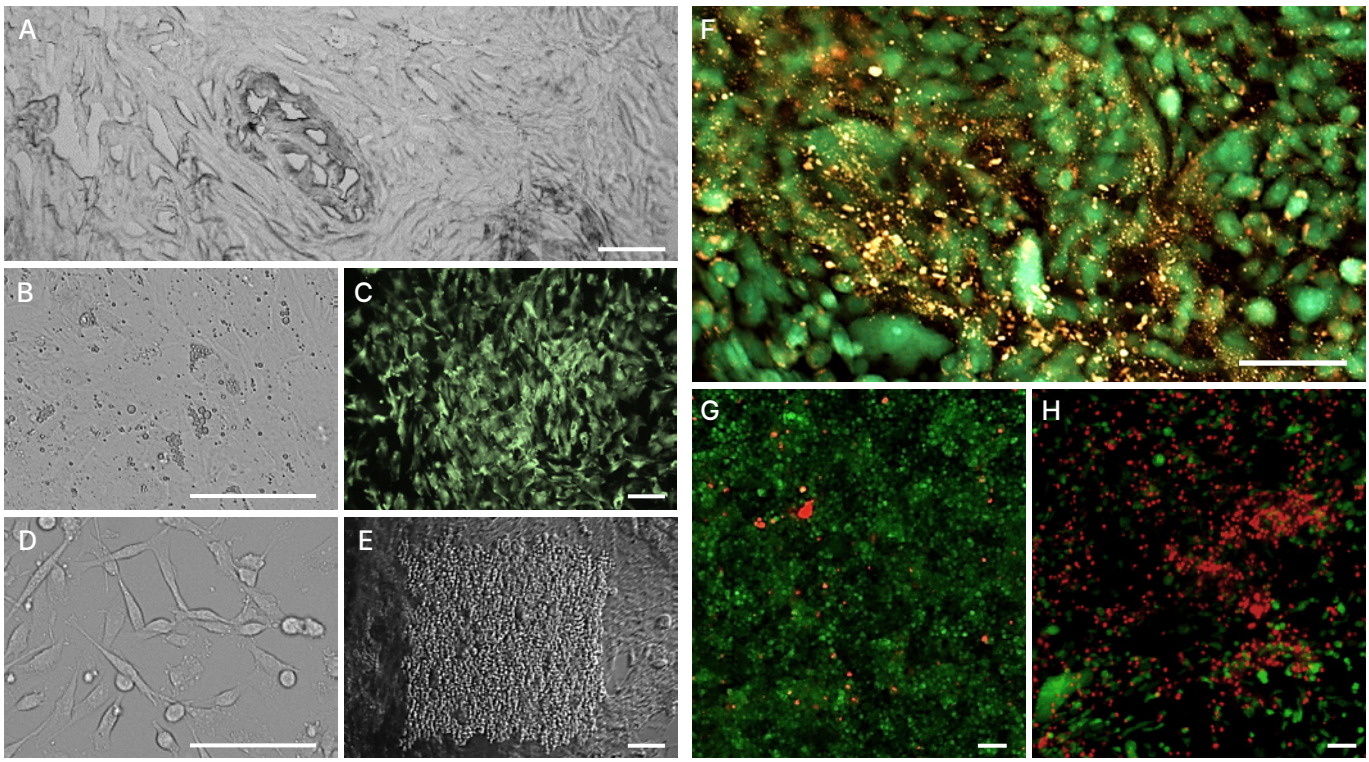

Figure 2. Tissue model preparation (A–E). (A) De-cellularized tissue section, (B) adipose and (D) TNBC cells were used to build a breast cancer model by printing (C) adipose cells followed by (E) TNCB cells onto the section, separated by 7 days of culture. Cells were stained with FDA. **Drug toxicity (F–H).** (F) The mature tissue was treated (G) with 4 µM Doxorubucin or (H) DMSO control for 72h and stained for live/dead cells. Panel F was stained with FDA (Green) and LipoSpot (Orange). Panel G and H were stained with FDA (Green) and PI (red). Scale-bar 100 µm.

Conclusion and discussion

The microenvironment is essential for cell behavior and tissue function. Here, we have successfully sectioned de-cellularized patient-derived breast cancer tumors and used the sections as a substrate for cell printing. The plane sections were suitable for micrometer positioning of the bioprinter printhead as well as binding cell attachment agent and cells via microfluidic printing. Importantly, cells propagated in the tissue sections to form highly dense cell patches seen in tissues. Thus, the tissue model represents a novel bioprinted in-vivo like model for cancer drug toxicity, cancer cell invasion, and advance co-cultures.

Methodology

Tissue sectioning and de-cellularization

De-cellularized human breast tumors were prepared by Fidelis/BioIVT 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-Na2 · 2H2O, 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-Na2 · 2H2O at 4 °C.

Tumors were sectioned frozen, placed on a glass objective, 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 % FBS HI (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 cell specific 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 (FluicellI) 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 5×106 cells/ml were printed onto the same position as the PLL and incubated for 5 days at 5 % CO2. Prior to printing MDA-MB-231 cells, the mature adipose cells were stained for 5 min with 8 µg/ml FDA (Fisher chemicals) and washed 3 times with PBS. The PBS was replaced with a co-culture

medium consisting of 75% differentiation medium (Promocell) and 25 % RMPI 1640 supplemented with 10% FBS HI (Gibco), and MDA-MB-231 cells were printed directly onto the tissue section (without prior printing of PLL) at a concentration of 10×106 cells/ml to the site of the previously printed adipose cells.

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 precision solutions for regenerative medicine, drug development and disease treatment. Our pipeline consits of cell-based therapeutics for type 1 diabetes treatment, high precision drug delivery technology for cancer treatment, advanced tissue-based cardiac screening models and innovative research instruments for single-cell biology and 3D bioprinting, based on proprietary microfluidic technology.

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