Validation of Bioprinted Vascularized Tumour Models for Anticancer Drug Evaluation

VoxCell BioInnovation

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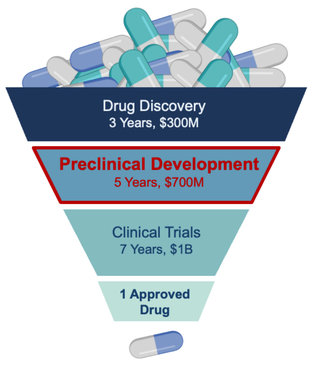
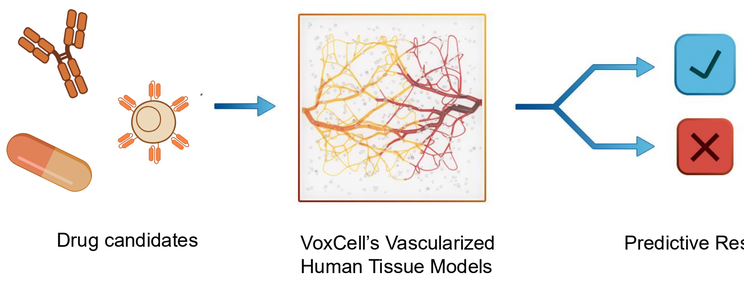
Introduction

Each year, the pharmaceutical industry spends billions of dollars on drug candidates that will fail.[1] *Many drugs fail due to a lack of translatable preclinical data that are predictive of clinical outcomes.*[2]



VoxCell's mission is to transform drug development with human-like vascularized tissue models:



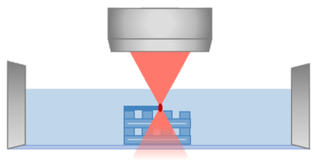
Drug candidates | VoxCell's Vascularized Human Tissue Models | Predictive Results

The **aim of this study** is to provide proof-of-concept data demonstrating VoxCell's bioprinted vascularized cancer models can generate reliable efficacy readouts using the clinically- approved cancer drug, Paclitaxel[3].

Materials

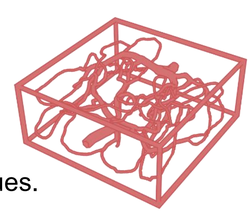
Modeling human cancer with VoxCell's proprietary technologies:

1. Two-Photon 3D Bioprinter



- Canada's first high-resolution bioprinter – 600x higher resolution than currently adopted bioprinting technologies.
- Two-photon polymerization with high accuracy and precision beam steering and motion control.

2. Vascularization Software



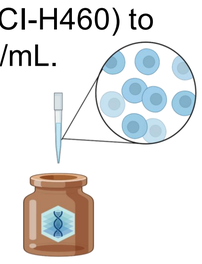
- Vascularization software captures the complex vascular structure and vessel diameters observed in real tumour tissues.

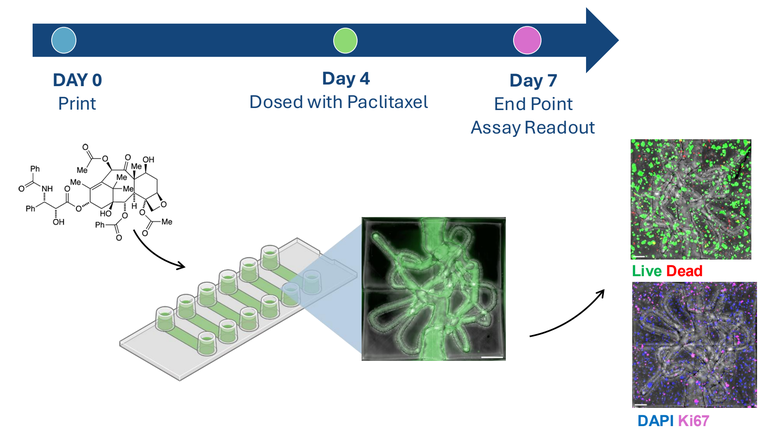
3. Tunable and Biocompatible Bioink

- Our customizable formulation of photo-crosslinkable semi-synthetic polymers provides mechanical characteristics and biocompatibility favorable to cell growth and proliferation.
- Mimics the extracellular matrix of tissues.
- Tissue stiffness customized to match that of tissue types in humans.

Methodology

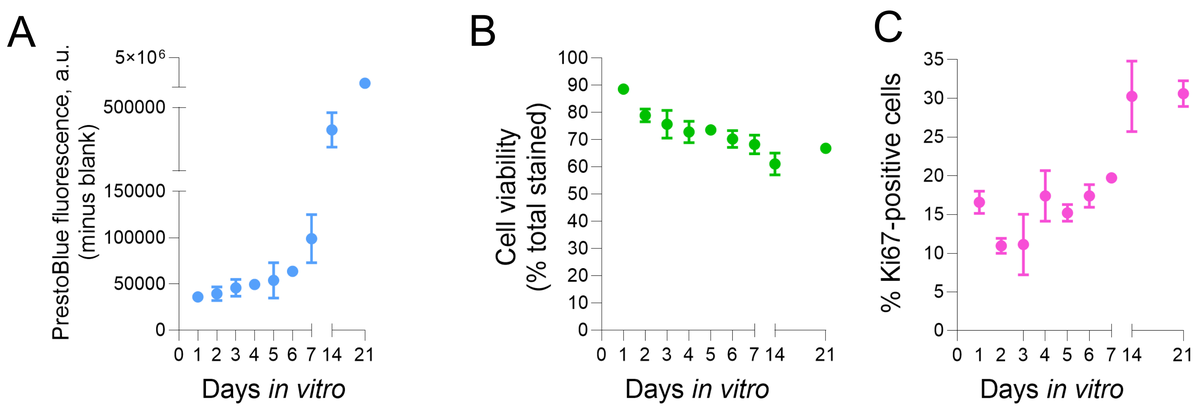
1. Use proprietary software to design tissue model with vascular network.
2. Add cancer cells (e.g., MDA-MB-231, NCI-H460) to bioink for final concentration of $5x10^6$ cells/mL.
3. Seed cell laden bioink into commercial perfusion chip from Ibidi GmbH, and perform high resolution 3D bioprinting.
4. Daily media change and monitoring of print stability up to three weeks.
5. Drug dosing and *in vitro* assays, directly inside perfusion chip.





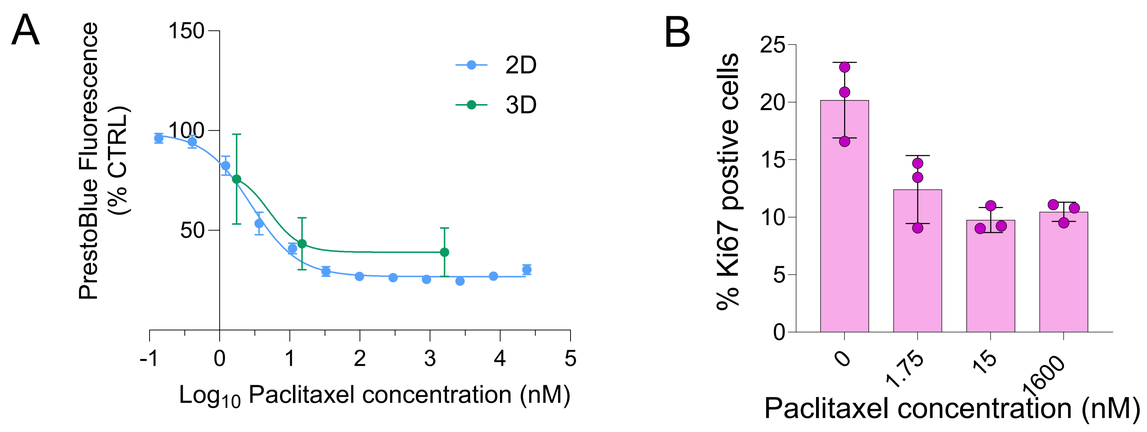
Middle: Visualization of vessel perfusion following FITC-dextran injection. Right: Representative images of Live/Dead (top) and Ki67 (bottom) fluorescent cell labeling in 3D printed tissue models. Scalebars = 200 µm.

Demonstration of Biocompatibility, Long-Term Cell Viability and Cell Proliferation in 3D Bioprinted Breast Cancer Model



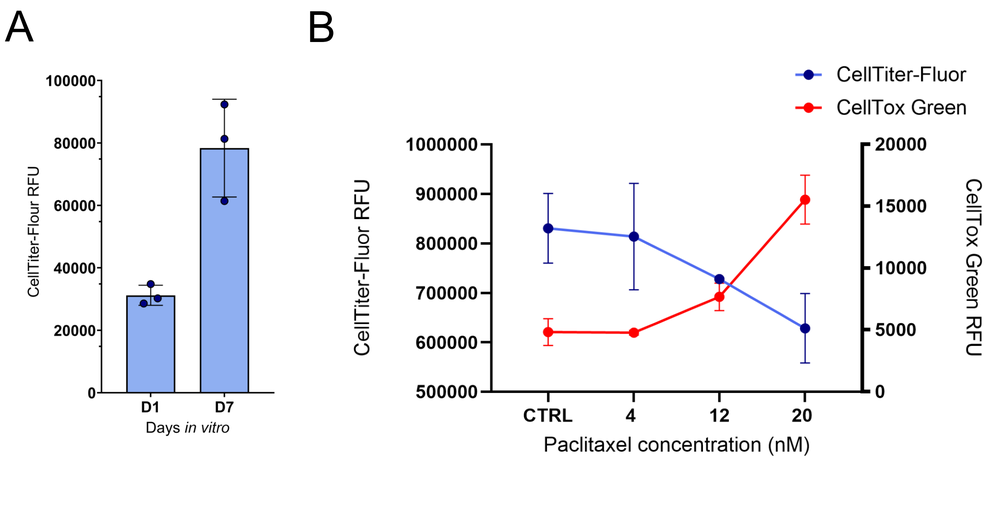
MDA-MB-231 triple-negative breast cancer cells were printed into 3D tissue models and cultured over 21 days inside perfusion chambers. Tissues showed increase in cell number and metabolic activity (PrestoBlue assay, **A**) with the proportion of live cells stabilized at ~70% (Live/Dead assay used to determine % Cell Viability, **B**), and upregulation in cell proliferation overtime (Ki67/DAPI staining, **C**). Each data point represents mean +/- SD of 4-6 technical replicates from 3 independent experiments. These 3D cell behaviour aligns with published data including spheroids generated using similar cancer cells [4] [5].

Dose-Dependent Response to a Clinically-Approved Anticancer Drug Replicated in 3D Bioprinted Breast Cancer Model



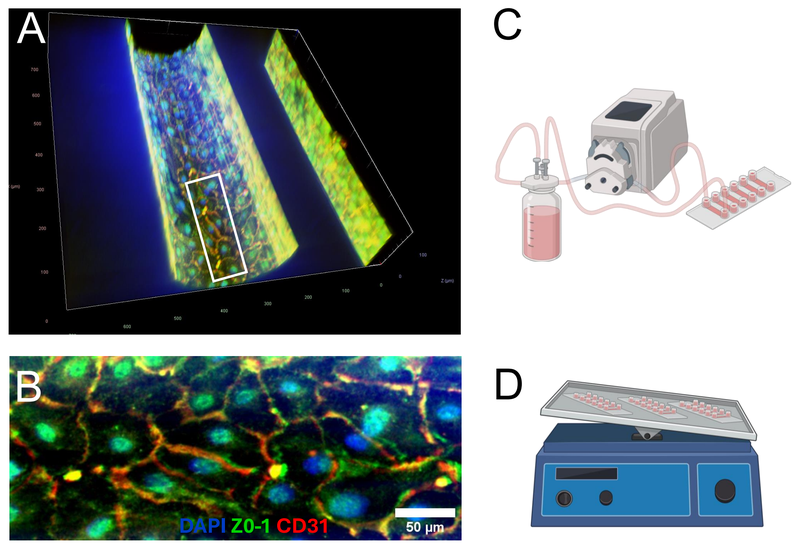
A, 2D IC_{50} for paclitaxel was estimated around 3.68 nM using the PrestoBlue cell viability assay, while assays performed on 3D tissue constructs suggest a concentration of 15 nM is required to reduce cell viability readout by 50% (points = mean +/- SD of 6-12 replicates per concentration from 2 independent experiments). **B,** Treated tissues were fixed after PrestoBlue readout and stained for cell proliferation marker Ki67 and nuclear dye DAPI. Paclitaxel reduced Ki67 expression in breast cancer tissue models in a dose-dependent manner, consistent with previous preclinical and clinical efficacy data on decrease in cell proliferation with this drug.[6]

Development and Validation of a Lung Cancer Model for External Partnership on Immunotherapy Screen



NCI-H460 non-small cell lung cancer cells were printed into 3D tissue models and cultured over 7 days inside perfusion chambers. Tissues showed increase in cell viability (CellTiter-Flour Assay, **A)** when comparing Day 7 (D7) readouts to Day 1 (D1)**. B,** Tissues demonstrated dose-dependent response to Paclitaxel with decrease in cell viability (CellTiter-Fluor) and increase in cell death (CellTox Green) associated with high Paclitaxel concentrations**.** Each data point represents mean +/- SD of 3 technical replicates. **C,** Representative 5X bright-field image of tumor cell aggregates and **D,** confirmation of active cell proliferation (Ki67+) contributing to the tumor spheroid formation. **E**, Representative 10X bright-field image with **F**, corresponding fluorescent image showing CD8+ T cell infiltration. CD8+ T cells were pre-labeled with CellTracker Green CMFDA. Scalebars = 200 µm.

Lining of Next Gen. Models with Endothelial Cells



A, The 3D bioprinted vasculature are lined with Human Umbilical Vein Endothelial Cells (HUVEC's) in VoxCell's next generation tissue models. HUVEC's are perfused into model, cultured under continuous flow for 7 days. **B,** ROI from A, demonstrating expression of characteristic endothelial cell markers. Flow can be achieved using a pump system (**C**) or rocker platform (**D**).

Conclusion and Next Steps

1. VoxCell's vascularization platform generates **scalable** tissue models with **perfusable** vascular-like structures that are stable and reproducible.
2. VoxCell's bioink provides the mechanical characteristics and **biocompability** favorable to cancer cell growth and proliferation.
3. Performance of VoxCell's cancer tissue model is **validated by cytotoxic testing** with standard of care therapeutics for cancer.

Up next: Expanding the complexity of our bioprinted models and recapitulation of *in vivo* cancer cell behaviour with the incorporation of additional cells (e.g., endothelial, stromal, and immune cells).

Learn more → www.voxcellbio.com/

References

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