Developing new therapies for primary liver cancer with precision bio-printed patient-derived organoids

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1 Introduction

Liver cancer is the third-leading cause of cancer-related deaths worldwide. Currently available systemic treatments have only modest efficacy and significant associated toxicity. Targeted, more effective therapeutic options are urgently required to ease the burden of disease. Lack of appropriate *in vitro* models is a major obstacle in drug development. Patient-derived organoid (PDO) technology may overcome limitations of conventional *in vitro* models. The reported rate of liver tumour PDO generation is low in non-defined matrices which do not accurately model the tumour microenvironment[1,2] and is a limiting factor in the full translational potential of liver cancer PDOs being realised[3].

2 Aim

1. To determine if the success of initial outgrowth of dissociated liver cancer tumour tissue can be improved by culture in defined bio-inks (Inventia Life Sciences) compared to traditional culture methods in non-defined matrices.
2. To develop a high throughput liver cancer PDO drug screening system using fully defined bio-inks developed by Inventia Life Science on the RASTRUM™ bio-printer[4].

3 Method

1. Percutaneous biopsy (33 patients) or resection (7 patients) specimens have been processed from 39 primary and one metastatic (lymph node) tumours (n=38 Hepatocellular Carcinoma (HCC), n=2 Cholangiocarcinoma (CCA)).
2. Tissues were dissociated and plated in Cultrex Basement Membrane Extract (BME2) using established protocols for PDO culture[1,2].
3. A subset of tissues were assessed in a pilot study to determine success of initial outgrowth in three novel matrices with 3 kPa (bio-inks A and B; unique peptide mix) or 1.1 kPa stiffness (bio-ink C; peptide mix = bio-ink B), compared to BME2.
4. Generated PDOs were bio-printed in 96 wells using the RASTRUM™ bio-printer (Inventia) in defined bio-inks and drug responses assessed, compared to a human immortalized hepatocyte cell line, PH5CH8.
5. We assessed 384 well bio-printing and drug responses in a CCA-PDO to determine suitability for high throughput (HTP) screening assays, quantified using high content imaging and analysis with a PerkinElmer Operetta CLS and PerkinElmer Harmony software.
6. We utilized the 384 well bio-printed model to assess drug responses in a CCA-PDO.

5 Conclusions

1. We have established a PDO bank from liver cancer patients that can be used for drug screening/repurposing experiments.
2. We show in a pilot study that liver cancer PDO derivation can be improved using bio-inks that more accurately model the native tumour environment than non-defined matrices such as BME2.
3. We have established HTP precision bio-printed primary liver cancer PDO models using the RASTRUM™ bio-printer in fully-defined conditions that can be used for assays in 96/384 well formats, in conjunction with technologies such as high content imaging.

6 Acknowledgements

Samples used in this study are part of the Perkins Cancer Biobank. We acknowledge the Western Australian Hospitals that support the Biobank and we are extremely grateful to generous participants who have provided their tissue.

7 References

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2 **Nuciforo S et al.** Organoid models of human liver cancers derived from tumour needle biopsies. *Cell Rep. 2018; 24 (5): 1363-1376*
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4 **Utama R.H et al.** A 3D bio-printer specifically designed for the high-throughput production of matrix-embedded multicellular spheroids. *iScience 2020; 23 (10): 101621*

8 Contact information

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4 Results

1. Primary liver cancer patient-derived organoid (PDO) establishment is improved by using defined bio-inks

Tumour type	Patient number	Sample type	Aetiology	BME2	A	B	C
CCA	2	Biopsy	HCV+ALD	-	+	+	+
HCC	8	Biopsy	NAFLD	-	-	-	-
HCC	9	Biopsy	ALD	+	-	-	-
HCC	16	Biopsy 1	HCV	-	+	+	+
		Biopsy 2		+	+	+	+
		Biopsy 3		-	-	-	-
HCC	17	Biopsy	ALD	+	-	+	+
HCC	35	Biopsy	ALD	-	-	+	-
HCC	36	Resection core	AT	-	-	-	-
		Resection periphery		-	+	-	-
HCC	37	Resection	HBV	+	+	+	+
HCC	39	Biopsy 1	HCV+ALD	+	+	+	+
		Biopsy 2		-	-	-	-
HCC	40	Biopsy	NAFLD	-	-	+	-
HCC	41	Biopsy	NAFLD+ALD	-	+	-	-

Table 1. Derivation of liver cancer PDOs in different 3D environments. Tumour tissue was dissociated using standard protocols and seeded in BME2, or defined Inventia bio-inks A (3 kPa), B (3k Pa) or C (1.1 kPa). Patient aetiology: Hepatitis B Virus (HBV) or Hepatitis C Virus (HCV), alcoholic liver disease (ALD), non-alcoholic fatty liver disease (NAFLD), Alpha-1-antitrypsin deficiency (AT).

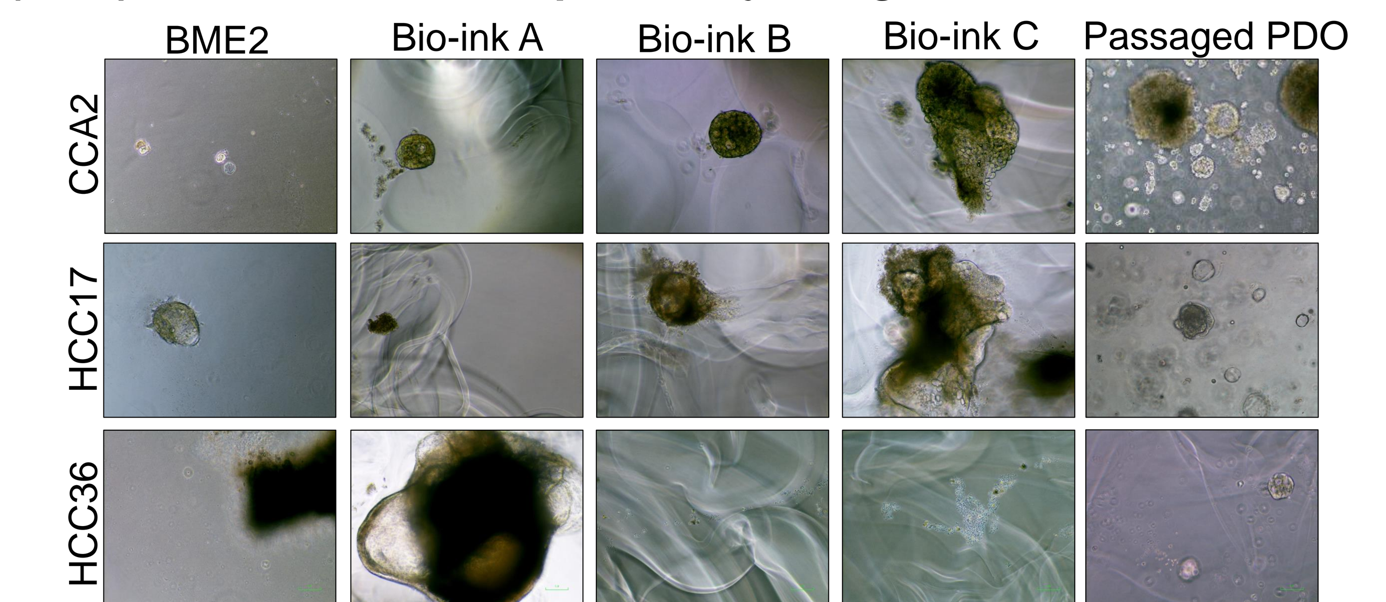

Figure 1. Examples of liver cancer PDOs derived in different 3D environments. *Brightfield images (100X magnification) show dissociated tumour tissue seeded in BME2, or defined Inventia bio-inks A (3 kPa), B (3k Pa) or C (1.1 kPa) and grown for 3 weeks, and derived organoid lines after passage*

2. Assessing growth of PDOs in Inventia bio-inks vs. BME2

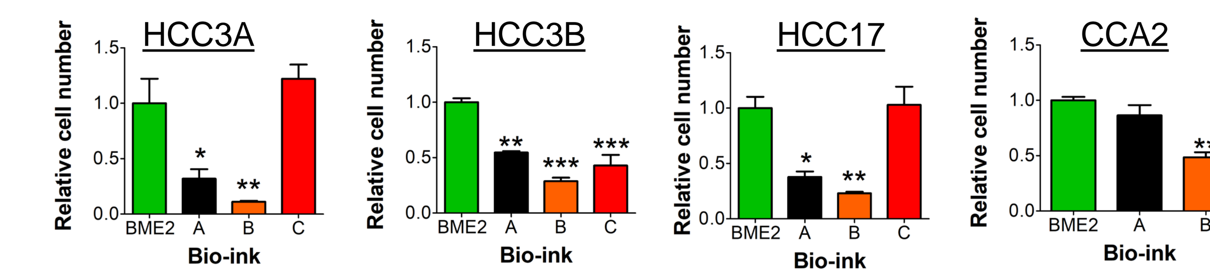

Figure 2. PDO matrix optimisation. *Established PDOs were bio-printed onto BME2, or defined Inventia bio-inks and grown for 7 days, and relative cell number assessed by CellTitreGlo3D assay. *p<0.05, **p<0.01, ***p<0.001 One way ANOVA with Tukey's post test. Data represents mean + SEM (n=3 to 14 wells).*

3. Sorafenib response of PDOs in Inventia bio-inks: 96 well model

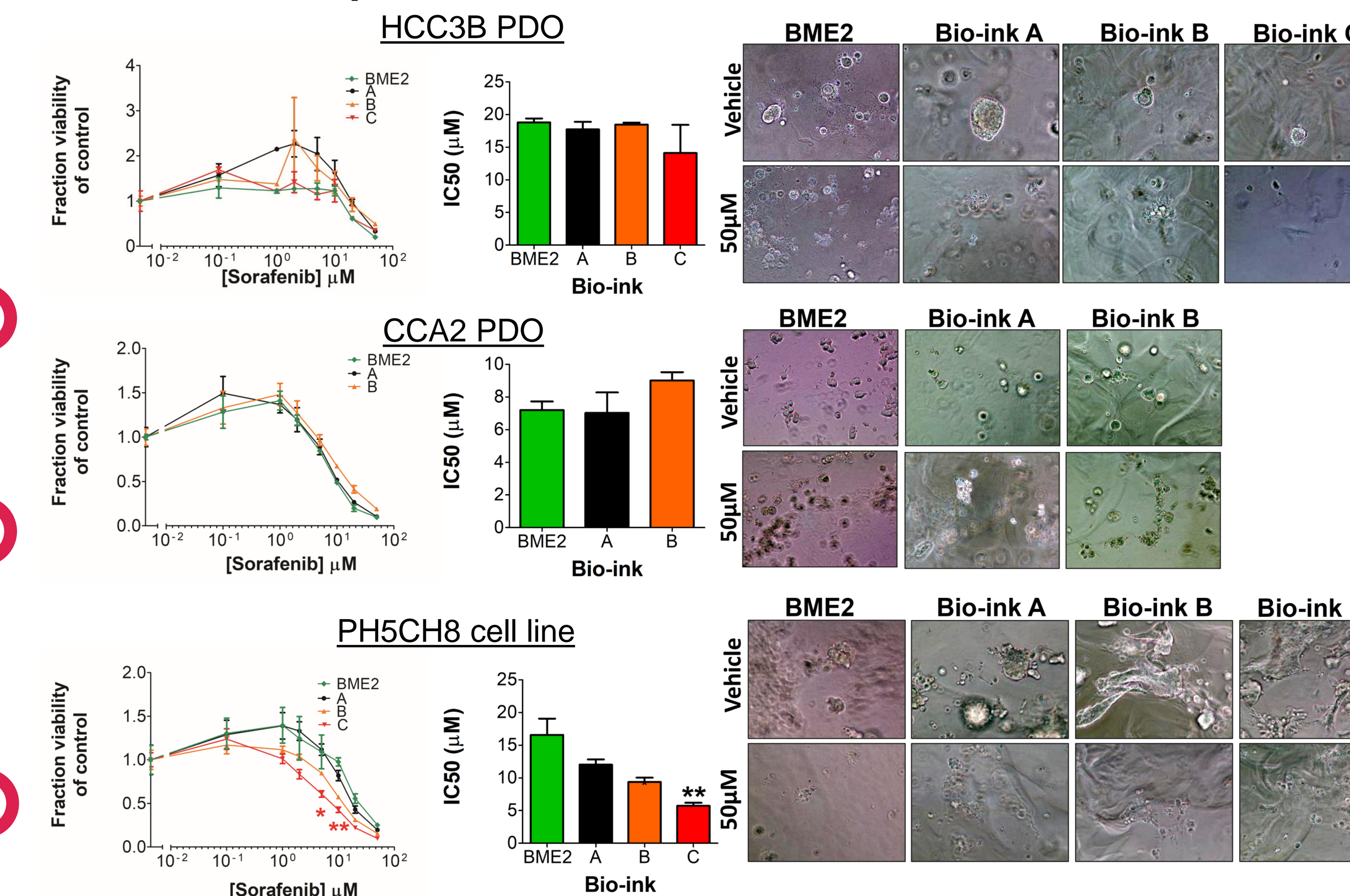

Figure 3. 96 well PDO bio-printing and Sorafenib response. *PDOs or a hepatocyte cell line (PH5CH8) were bio-printed onto BME2*, or defined Inventia bio-inks and treated with Sorafenib 24 hours post-printing. Relative cell number assessed by CellTitreGlo3D assay and compared to vehicle controls. IC50 was calculated in each condition. *p<0.05, **p<0.01, One way ANOVA with Tukey's post test (IC50 data) or two way ANOVA with Bonferroni post test (Drug treatment data). Data represents mean ± SEM (n=3 wells). Brightfield images were taken immediately prior to viability assay at 200X magnification.

4. Optimising a HTP 384 well bio-printed PDO model

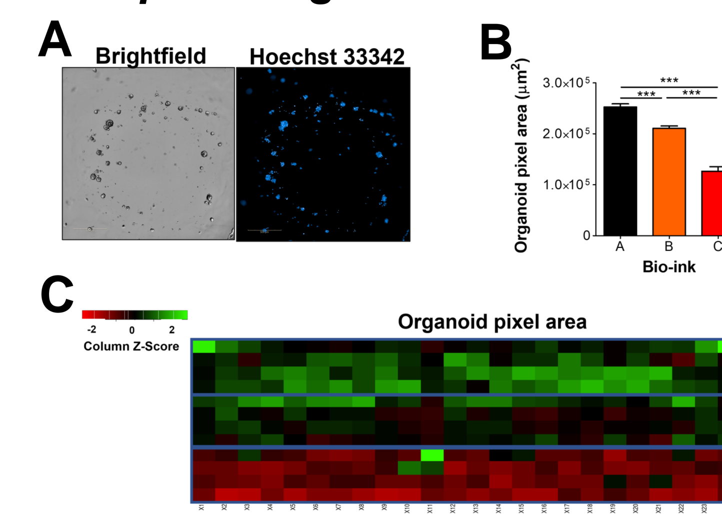

Figure 4. Developing a 384-well bio-printed PDO model. *Bio-printing of CCA2 in Inventia bio-inks in 384 HTP format was undertaken. 1000 cells/well were printed and grown for 7 days, nuclei stained with Hoechst 33342 and imaged using a PerkinElmer Operetta CLS and analysed by texture-based recognition of organoid areas with Harmony software. (A) Brightfield and fluorescence images (5X objective) were captured. (B) Image-based analysis to determine organoid area per well. (C) Heatmap shows distribution of organoid area. ***p<0.001, One way ANOVA with Tukey's post test. Data represents mean + SEM (n=96 wells).*

5. CCA2 drug response in 384 well bio-printed BME2 vs. Bio-ink A

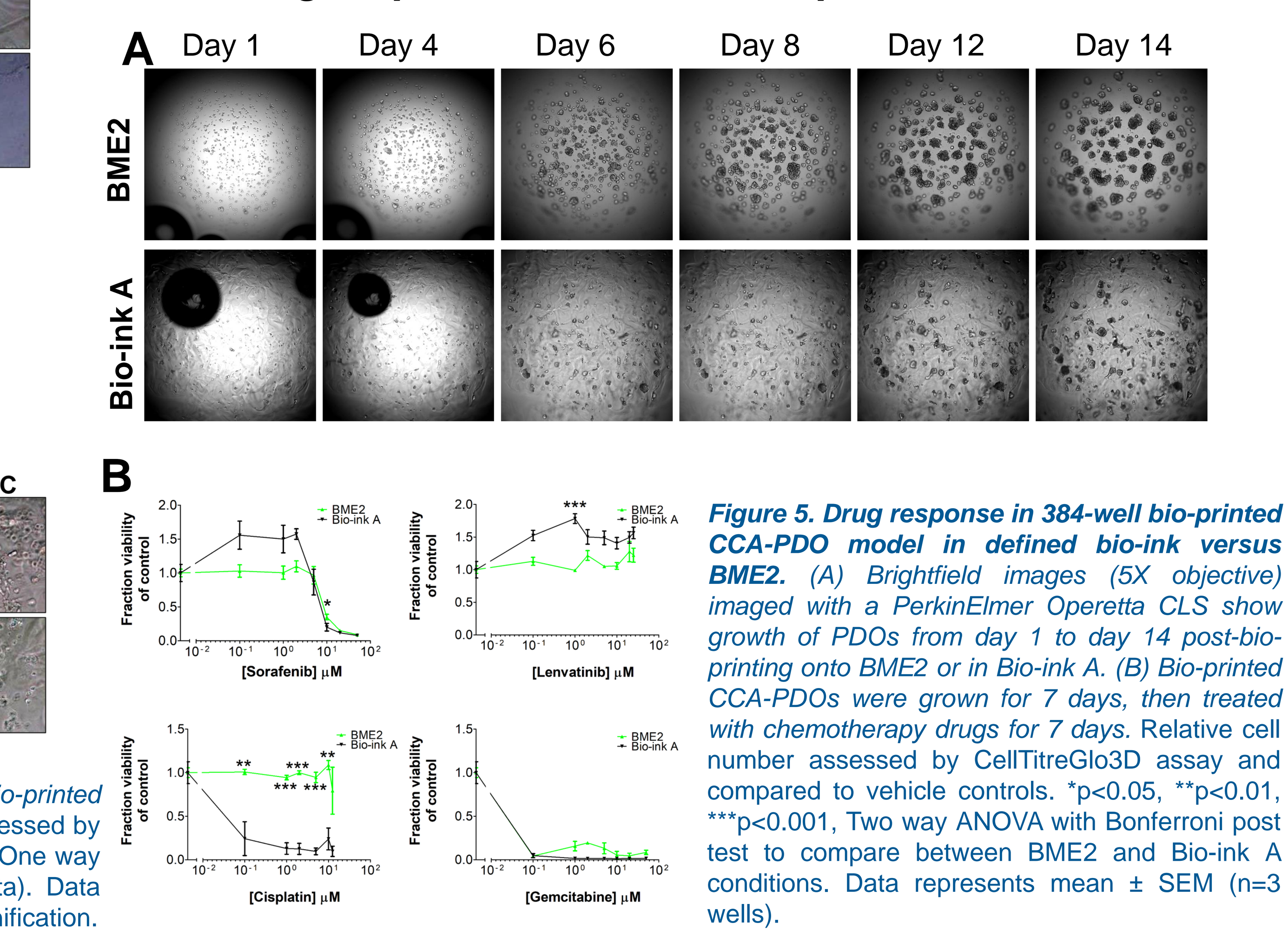

Figure 5. Drug response in 384-well bio-printed CCA-PDO model in defined bio-ink versus BME2. *(A) Brightfield images (5X objective) imaged with a PerkinElmer Operetta CLS show growth of PDOs from day 1 to day 14 post-bio-printing onto BME2 or in Bio-ink A. (B) Bio-printed CCA-PDOs were grown for 7 days, then treated with chemotherapy drugs for 7 days.* Relative cell number assessed by CellTitreGlo3D assay and compared to vehicle controls. *p<0.05, **p<0.01, ***p<0.001, Two way ANOVA with Bonferroni post test to compare between BME2 and Bio-ink A conditions. Data represents mean ± SEM (n=3 wells).

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