ONCO-Chip^{3D}: facilitating physiologically relevant drug and cell therapy screening in complex 3D tumour models

K. Paterson[1], J. Beinarovica[1], A. Sim[1,2] and M. Zagnoni[1,3]

[1] ScreenIn3D, Glasgow, United Kingdom

[2] AMS Biotechnology (Europe) Ltd, Abingdon, United Kingdom

[3] University of Strathclyde, Glasgow, United Kingdom

contact: info@screenin3d.com

Acknowledgements

Lisa Neilson*, Emily Kay*, Joanne Edwards#, Sara Zanivan*, Chiara Braconi#

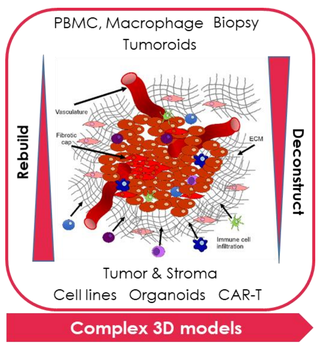
* *Tumour Microenvironment and Proteomics, Cancer Research UK Beatson Institute*

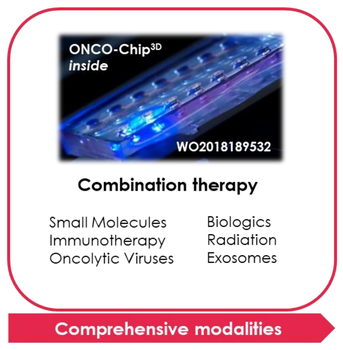
Institute of Cancer Science, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK

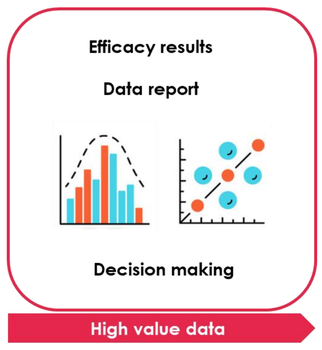
SCREEN IN 3D

Introduction

- Cell behaviour is altered in two-dimensional (2D) cultures with respect to the native 3D *in vivo* microenvironment (Breslin & O'Driscoll 2013), which affect cellular behavior and drug responses (Tung et al. 2011; Pickl & Ries 2009).
- Complex 3D *in vitro* cancer models based on the use of patient-derived cells can facilitate immunotherapy screening and combination studies in a physiologically relevant context.
- Microfluidic technologies offer precise control over the tumor microenvironment, and require only a fraction of reagents and cells, whilst enabling an increase in throughput even when small samples are available.
- ScreenIn3D's microfluidic technology and associated protocols enable the generation, culture and screening of hundreds of 3D complex cancer models from a mixture of primary cell lines (e.g. spheroids and organoids) and biopsy tissue (e.g. tumoroids), providing at least 20X more data throughput for the same amount of material used.
- Our proprietary ONCO-Chip^{3D} technology uses a self-generated perfusion and drug concentration gradient to miniaturise the screening of limited cellular material (e.g. biopsy-derived 3D models).

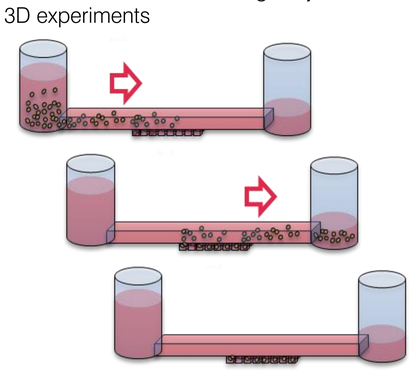




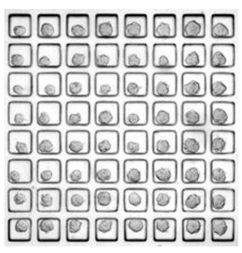


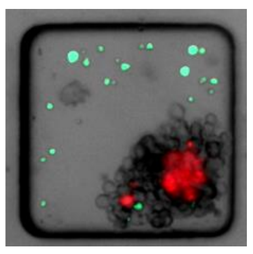
Our technology

- As low as 1,000 cells in a single injection for tens of 3D experiments

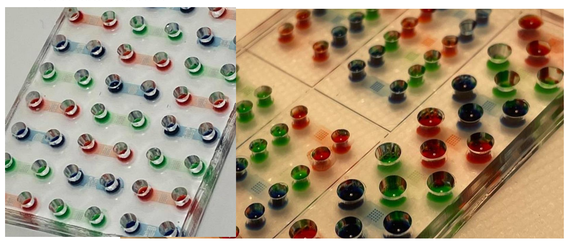


- Multiple cell types can be inserted to increase model complexity at desired time points

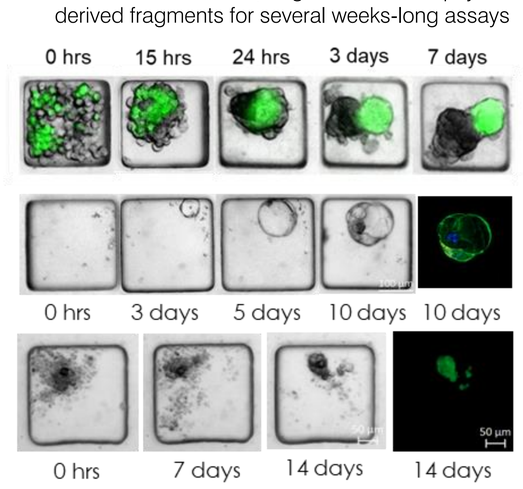




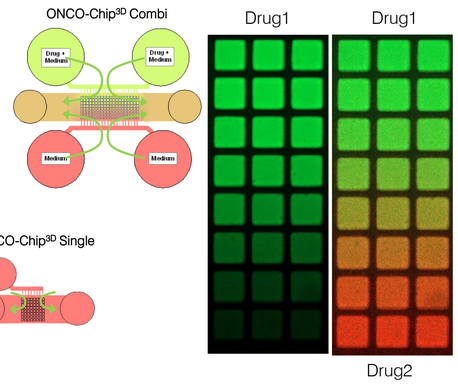
- Shear-stress free perfusion is achieved without any external flow actuation



- 3D co-culture models, organoids and biopsy-derived fragments for several weeks-long assays



- Controllable and long-lasting molecular concentration gradients are generated to miniaturize combination studies and quantify dose response analysis

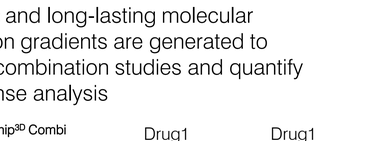


Readouts

- Cell specific response

- 3D structure markers

- Real time monitoring

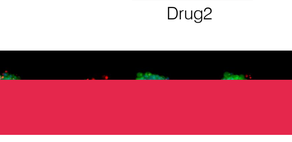


- Phenotypic characterization
- Growth rate
- Live/dead
- Immunofluorescence
- Supernatant profile
- Sample recovery and off-chip processing

- Morphology, such as disaggregation or compaction (Kelm et al. 2003).

$$SF = \frac{P^2}{4\pi A}$$

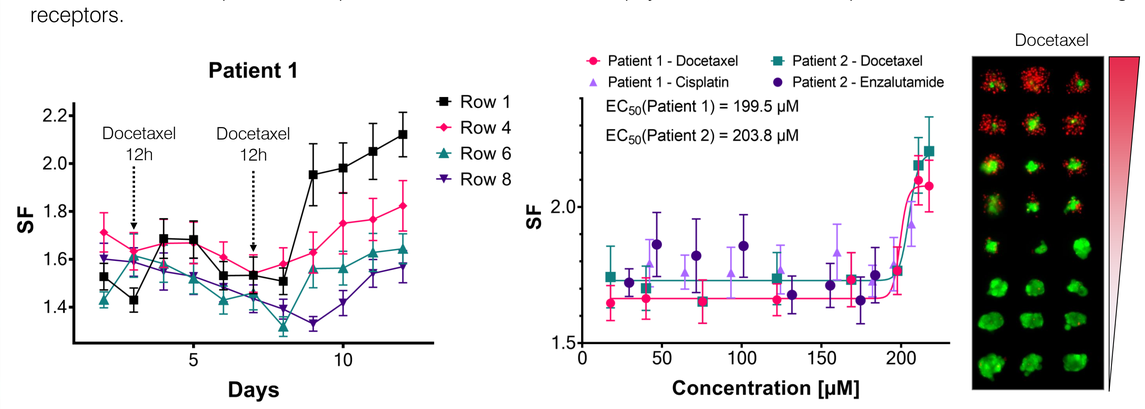
- Measure of drug efficacy using multiple parameters.



$$VF = \frac{Area_{+FDA}}{Area_{BF\_initial}}$$

Drug concentration gradients to screen biopsy-derived tumoroids

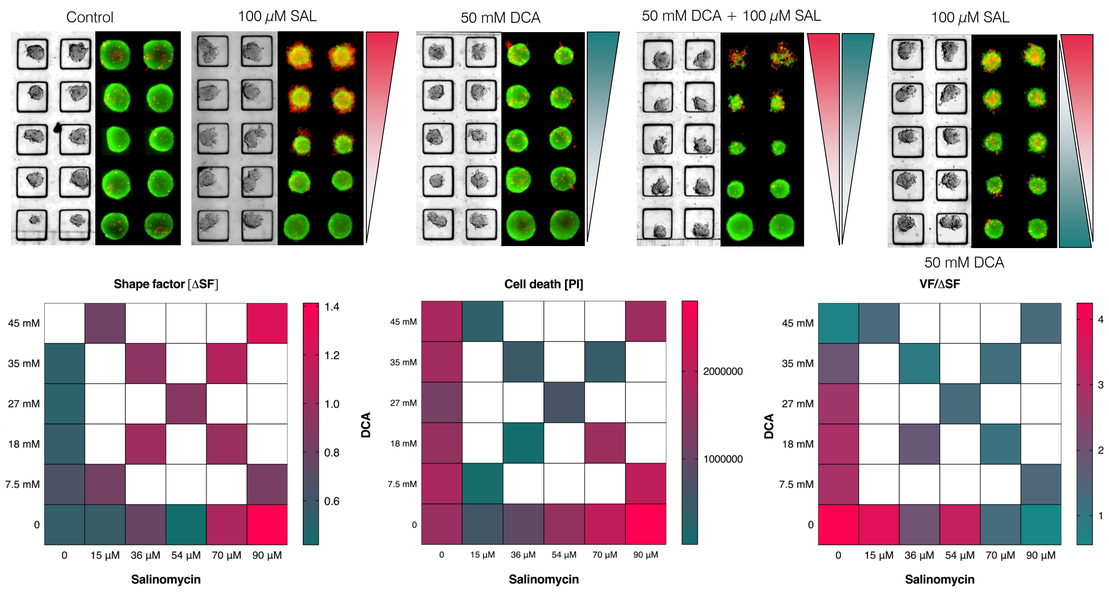
- Fractionated chemotherapy was mimicked using biopsy-derived tissue, showing a concentration-dependent response to Docetaxel, but not cisplatin.
- RT-PCR showed the presence of prostate cancer cells in the biopsy core, as well as the presence of functional androgen receptors.



Protocols as described in: Mulholland et al. (2018) *Scientific Reports*

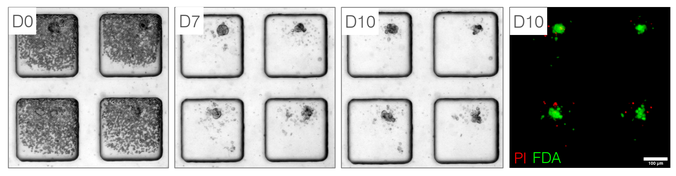
Drug concentration gradients for combination drug screening

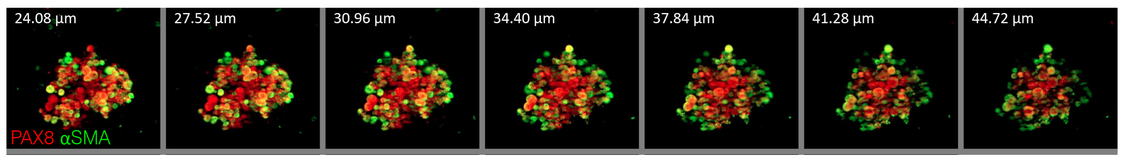
- Dichloroacetate (DCA) and salinomycin (SAL) were reported to exert a synergistic effect in spheroids derived from HCT116 cells ((Skeberdytė et al., 2018).
- Colorectal HCT116 cells were seeded into the device and allowed to aggregate for 24 hours.
- On day 1, drug concentration gradients were initiated and maintained for 16 hours, whilst the devices were placed in the incubator.
- 3 days later viability staining was conducted using FDA and PI.



Primary tumor fragment culture and characterization

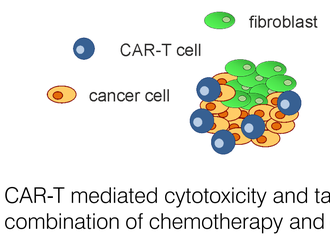
Fragments of high grade serous ovarian tumour resections were seeded into the ONCO-Chip^{3D} and maintained in culture for 10 days, when viability staining using PI and FDA was performed.

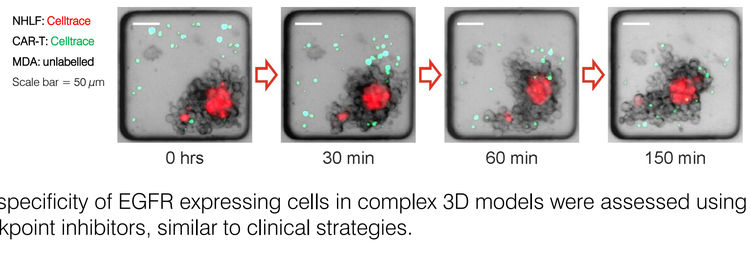




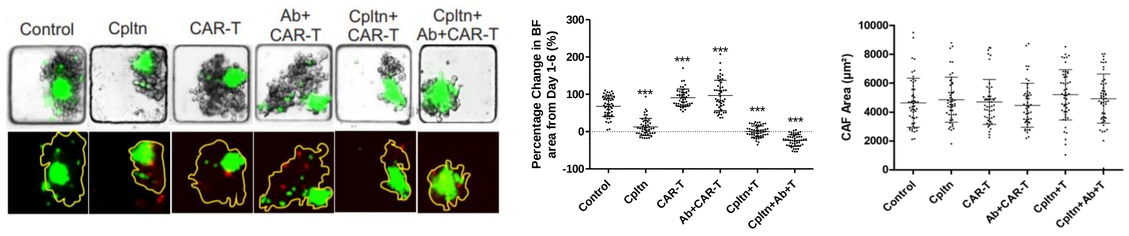
To demonstrate the presence of tumour cells (PAX8) and cancer-associated fibroblasts (αSMA) on day 10, the fragments were fixed in situ and stained using immunofluorescence.

Cell therapy efficacy studies





CAR-T mediated cytotoxicity and target specificity of EGFR expressing cells in complex 3D models were assessed using combination of chemotherapy and checkpoint inhibitors, similar to clinical strategies.



amsbio

University of Strathclyde Glasgow

Why use SCREEN IN 3D services?

HUMAN TME | COST EFFECTIVE | COMBINE | DESIGN | PERSONALISE

References

Breslin, S. & O'Driscoll, L., 2013. Three-dimensional cell culture: the missing link in drug discovery. Drug discovery today, 18(5–6), pp.240–9.

Kelm, J. M., Timmins, N. E., Brown, C. J., Fussenegger, M. & Nielsen, L. K., 2003. Method for generation of homogeneous multicellular tumor spheroids applicable to a wide variety of cell types. Biotechnol. Bioeng. 83, 173–180.

Mulholland, T. et al. 2018. Drug screening of biopsy-derived spheroids using a self-generated microfluidic concentration gradient. Scientific Reports, 8 (1), 14672

Pickl, M. & Ries, C.H., 2009. Comparison of 3D and 2D tumor models reveals enhanced HER2 activation in 3D associated with an increased response to trastuzumab. Oncogene, 28(3), pp.461–468.

Ruppen, J. et al., 2015. Towards personalized medicine: chemosensitivity assays of patient lung cancer cell spheroids in a perfused microfluidic platform. Lab Chip, 15(14), pp.3076–3085.

Skeberdytė, A., Sarapinienė, I., Aleksander-Krasko, J., Stankevičius, V., Sužiedėlis, K., & Jarmalaitė, S. (2018). Dichloroacetate and Salinomycin Exert a Synergistic Cytotoxic Effect in Colorectal Cancer Cell Lines. Scientific Reports, 8(1), 1–13. https://doi.org/10.1038/s41598-018-35815-4

Tung, Y.-C. et al., 2011. High-throughput 3D spheroid culture and drug testing using a 384 hanging drop array. The Analyst, 136(3), pp.473–478.