Evaluating anticancer agents in a 3D pancreatic cancer cell culture model.

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Introduction

Current chemotherapeutic regimes for pancreatic cancer have failed to provide any substantial clinical benefits, with a 5 year survival rate of less than 5%. The failure of classic cytotoxics has led to the clinical development in recent years of targeted agents against validated signalling pathways. However, so far the approach of targeted agents alone or in combination with chemotherapeutics has failed to improve the prognosis of pancreatic cancer patients. There is currently an urgent need for more efficient models to improve drug discovery programs and gain greater insights into the complexity of tumour biology.

AIMS: To evaluate the predictive potential of a miniaturised 3D pancreatic cancer cell culture model against a selection of anticancer drugs compared to traditional 2D drug discovery culture models on various platforms.

The predictive utility of traditional cell models, such as two-dimensional cell monolayer cultures may be limited particularly in pancreatic cancer drug discovery.

Pancreatic tumours have a unique morphology amongst solid tumours of a dense and poorly vascularised stroma. To explore the predictive potential of a more complex cell model system in pancreatic cancer, we developed a three-dimensional (3D) micro tumour assay. This miniaturised 3D cell culture assay utilises a biological hydrogel based extracellular matrix for 3D growth on a 384 well microplate. Suitability for use in a high-throughput screening campaign was assessed and activity of known compounds was compared verses monolayer culture methods.

The 3D culture assay presented here attempts to address many of the current challenges in automating more complex culture models for use in drug discovery and high content analysis programs: the generation of reproducible and consistent homogenous 3D cultures, the utilisation of standard lab plasticware and conventional imaging platforms or plate readers and automated image analysis of 3D cultures.

Materials & Methods

A panel of pancreatic cancer cell lines were seeded on growth factor reduced Matrigel in 384 well imaging plates for 3D cultures. Plates were incubated in standard conditions for 72 hours until 3D micro tumour structures had formed, media was then changed with compound addition. Media and compounds were replaced every 48 hours for a further 6 days. All drug additions and media changes were performed on the Agilent Bravo automated liquid handler. Resazurin and Calcien AM were added using optimised protocols and plates were read on either the Perkin Elmer Envision or Operetta.

Image analysis was performed by the Harmony software linked to the Operetta using standard intensity measurement scripts.

Semi-automated assay platform

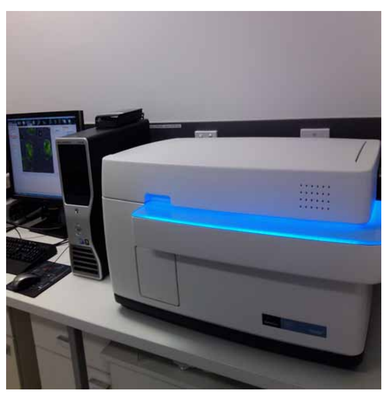
Compound addition, staining, media change

Agilent Bravo™

Fluorescent read for resazurin assay

Perkin Elmer Envision™

Imaging platform for morphology analysis



Perkin Elmer Operetta™

Results

Cell viability was determined by an optimised resazurin assay for both 2D and 3D cultures. IC_{50} values for a selection of anticancer compounds are compiled below (Table 1.).

	AsPc-1		BxPc-3		Panc-1	
	2D	3D	2D	3D	2D	3D
Doxorubicin	0.1563	0.4405	0.0552	0.6165	0.0656	0.3088
Vinorelbine	0.0270	0.0592	0.0160	0.0900	0.0267	0.0823
Epirubicin	0.1376	0.3100	0.0544	0.6401	0.0542	0.2481
Gemcitibine	0.0081	0.0292	0.0069	0.0277	0.0248	0.0764
Docetaxel	0.0033	0.0172	0.0011	0.0039	0.0020	0.0134
Paclataxel	0.0047	0.0345	0.0011	0.0179	0.0056	0.0423

Table 1. IC_{50} values (µM) determined by resazurin cell viability.

Several drugs displayed statistically significant resistance effects in the 3D cultures, particularly the taxanes and doxorubicin. A dose curve shift and reduction in maximum inhibition effect was seen in gemcitibine and vinorelbine.

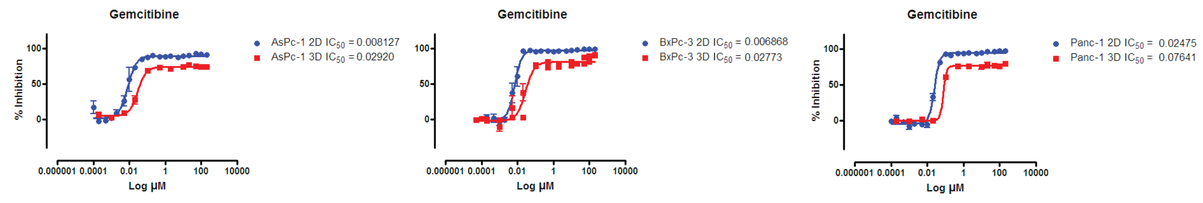


Figure 1. Gemcitibine dose response curves in 2D vs 3D cultures (resazurin assay).

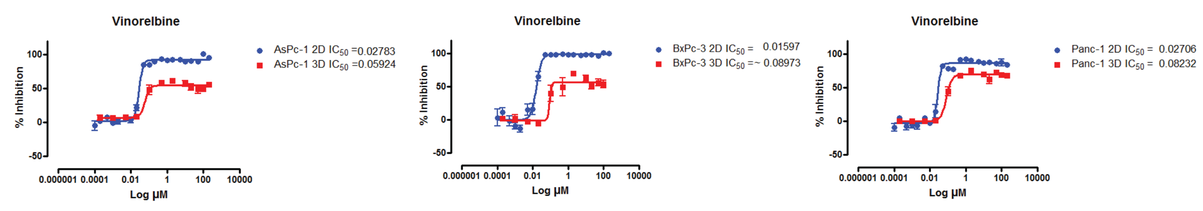


Figure 2. Vinorelbine dose response curves in 2D vs 3D cultures (resazurin assay).

To confirm resazurin viability results in 3D cultures and evaluate imaging platforms, we compared results obtained from fluorescent microscopy imaging using a live cell stain. Figures 3 and 4 show examples of the live stain overlayed on the brightfield image of each well of a dose response of a drug. Analysis of the in-focus z-section using a range of parameters such as intensity and total area was used in the Harmony analysis software to determine IC_{50} values.

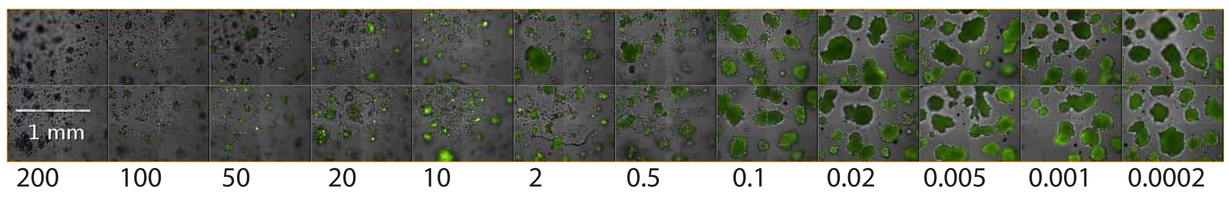


Figure 3. µM concetration of Doxorubicin in Panc-1 3D culture and effects on spheroid structure.

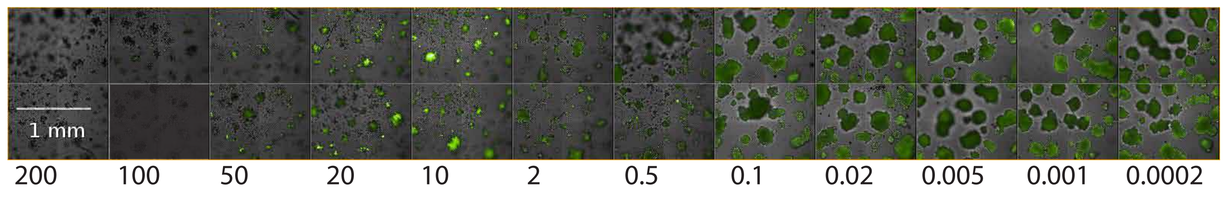


Figure 4. µM concetration of Epirubicin in Panc-1 3D culture and effects on spheroid structure.

	Panc-1	
	Resazurin	Calcien AM
Doxorubicin	0.3088	0.2995
Vinorelbine	0.0693	0.0231
Epirubicin	0.2481	0.3115
Gemcitibine	0.0764	0.1165
Docetaxel	0.0134	0.0517
Paclataxel	0.0423	0.0892

Table 2. Comparison of IC_{50} values from both viability methods.

Conclusions

The 3D cell viability assay examined here demonstrates it is possible to generate a reproducible, cost effective 3D culture system for use in future high content analysis applications, using standard lab consumables and automated imaging equipment. The adoption of 3D culture systems for use in drug discovery programs will require further automation and validation. However, this culture system may ultimately provide better predictive data on novel compounds and insights into pancreatic cancer tumour penetration of current chemotherapy drugs.