Pharmacokinetic profiles revisited in 3D microfluidic tumour models

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Introduction

The efficacy or toxicity of a drug is dependent on the concentration at the target. This concentration varies with time owing to absorption, distribution, metabolism and excretion, resulting in a pharmacokinetic (PK) profile. Understanding the relationship between PK, pharmacodynamics (PD) and efficacy is critical to the successful development of new medicines (1). At present this relationship is primarily investigated using animals, this is time-consuming, ethically undesirable and prone to a lack of translation, particularly if animal and human PK differ significantly. This contributes to the low success rate of oncology medicines in the clinic.

At present there are a lack of *in vitro* alternatives to explore this relationship. In animals and humans drug concentration varies with time, whereas *in vitro* experiments are performed at fixed concentrations. This limits the translational relevance of the *in vitro* experiments (2).

To overcome this issue we have developed a microphysiological system (MPS) that is able to explore PK/PD efficiency relationships on 3D tumour models/organoids, following mono or combination therapy. The MPS creates a PK profile by periodically changing the concentration of drug in each well, exposing the 3D tumour model to a PK-like profile. This unique combination of *in vitro* PK profile drug exposure and 3D tumour models promises to improved translatability and clinical success rates of novel oncology therapies.

Aims

1. Demonstrate that our MPS is able to generate PK profiles for monotherapy and combinations of 2 anti-cancer drugs.
2. Demonstrate MPS's ability to mimic anti-cancer treatment regimens on complex 3D tumoroids, and assess treatment efficacy and PK/PD relationship.

Methods

All experiments were carried out using CN Bio's PhysioMimix™ PK MPS device and controller. PK profiles were inspired from literature data (3, 4) and adapted using MPS editor software.

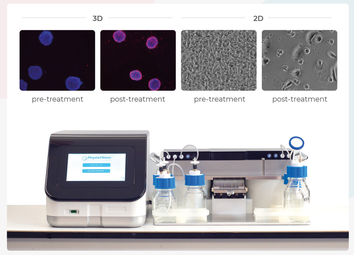
Tumour cell lines used were A549 (non-small lung carcinoma, NSCLC) and SW620 (colorectal carcinoma) conditioned mostly as 3D tumoroids encapsulated in Matrigel® droplets to better mimic a tumour microenvironment. A 2D monolayer was used in a single experiment for comparison reasons.

The A549 cell line was maintained and proliferated in RPMI1640. The SW620 cell line was maintained and proliferated in L-15 (Leibowith?). Media composition, cell passages and tumoroid formation followed standard protocols. Vehicle control consisted of cell line specific maintenance medium containing 0.1% DMSO.

3D tumoroids were collected 48h post seeding, transferred to 24- well plates and encapsulated in Matrigel droplets (2 droplets per well). Plates were placed in the MPS device using a proprietary lid prior to microfluidic experimental onset.

At the end of the experiment, cells were imaged using a Nikon Eclipse Ti2 inverted microscope. Images were analysed by customized macros in Fiji ImageJ (5). Cell viability was assessed using the CellTiter-Glo® 3D Cell Viability assay (Promega). pAKT expression was assessed by AKT (Phospho) [pS473] Human ELISA kit (ThermoFisher).

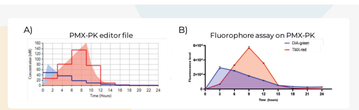
Figure 1 – The MPS device mimics *in vivo* PK profiles



A: PhysioMimix-PK MPS controller and PK generator device can treat 2D and 3D tumour models.

B: MPS is able replicate/programme PK profiles *in vitro* in a 24-well standard plate format. PK profiles are designed using bespoke device software.

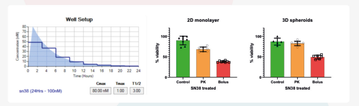
Figure 2 – The MPS device recapitulates mono- or combo- drug profiles



A: Representation of MPS software designed PK profiles for 2 drugs added to single culture well.

B: Measured PK profiles for 2 drug-equivalents, for 24h with 1 h timepoints.

Figure 3 – Mimicking *in vivo* drug exposure in 3D tumoroids prevents overprediction of efficacy in traditional 2D cultures



SW620 colorectal tumours were treated with topoisomerase inhibitor (SN38) for three days. 2D and 3D tumours were treated with either a PK profile mirroring murine *in vivo* exposure (equivalent to 500 mg/kg every 24h for 72 h) or bolus exposure at 80 nM (continuous drug exposure – traditional *in vitro* approach).

Figure 4 – Demonstrating PK/PD/efficacy relationship *in vitro* for a PI3Kα inhibitor treatment of lung tumours using the MPS device

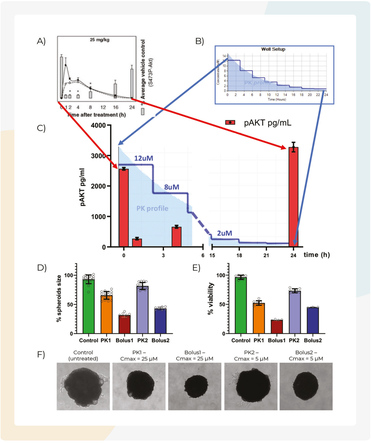


Figure 5 – Comparing combination drug dosing regimens for colorectal tumours *in vitro* using the MPS device

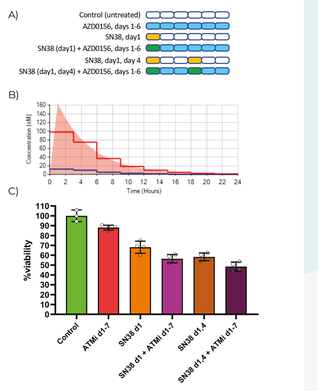


Figure 4 continued...

PK/PD/efficacy relationship was assessed for PI3K inhibitor BYL719 treatment of NSCLC cells (A549).

A: PK/PD relationship in mice for BYL719 and p-AKT in Rat1-myr-p110α tumours as determined by Fritsch et al. (3).

B: BYL719 PK profile, applied to A549 tumoroids using the MPS (equivalent to 50 mg/kg murine oral dose) for 24h – cellular samples taken at regular intervals to assess **(C)** cellular p-AKT levels. **(D-F)** Equivalent tumoroids

Figure 5 continued...

SN38 (topoisomerase inhibitor) monotherapy and combinations with a DNA-damage response inhibitor (ATMi) efficacy on SW620 3D tumoroids was assessed using the MPS device.

A: SN38/ATMi treatment regimens were designed to recapitulate *in vivo* experiments (4).

B: PK profiles for SN38 (Cmax = 20 nM) and ATMi (Cmax = 162 nM), applied on SW620 3D tumoroids, using our MPS, for 6 days were adapted from literature equivalents (SN38 Cmax equivalates 150 mg/kg murine oral dose of Irinotecan; ATMi Cmax equivalates 150 mg/kg murine oral dose).

C: Endpoint viability for the SN38/ATMi monotherapy and combinations using PK profiles displayed on **(B)** and regimens described in **(A)**. Various treatment schedules displayed different results, consistent with literature data (4).

Conclusion

Pharmacokinetic profiles (PK) and their relationships with pharmacodynamics (PD) and efficacy were assessed for two anti-cancer drugs on 3D tumoroids derived from NSCLC and colorectal carcinoma cell lines, using a newly developed MPS. Not only was our MPS able to recapitulate human/animal PK profiles but it also demonstrated to ability to accommodate 2D and complex 3D biological constructs in a 24-well plate format. Most experiments were carried on Matrigel-encapsulated tumoroids representing 3D cell constructs with additional stimulation/cues from the microenvironment, a feature known to enhance physiological relevance. SN38 monotherapy treatment of SW620 colorectal tumour models confirmed that mimicking *in vivo* drug exposure induces different responses in 3D versus 2D cell cultures and showed traditional bolus dosing over predicts efficacy, particularly on 2D cell cultures. More significantly the *in vivo* PK/PD/efficacy relationship, could be recapitulated by dosing a PI3Kα inhibitor, with a PK-like profile, onto A549 NSCLC tumoroids, demonstrating the translational relevance of the MPS.

Lastly, to explore combinations and scheduling, topoisomerase inhibitors and DNA-damage response inhibitors were combined in various dosing regimen on colorectal tumoroids, results obtained were consistent with literature (4). This demonstrates the utility of the MPS for combination therapies, an area in which is notorious for requiring large, costly animal studies. Thus, the MPS is able to recapitulate both anti-tumour drug efficacy (monotherapy or combinations), and PK/PD relationships, following various regimens and PK profiles on 3D tumoroids. The MPS is a tool with the potential to create data with high translational value, allowing a detailed understanding of the PK/PD/efficacy relationship which could previously only have been obtained from animal models.

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