Use of Pu·MA System® 3D for Single Spheroid Assays with Downstream Metabolomics

Introduction

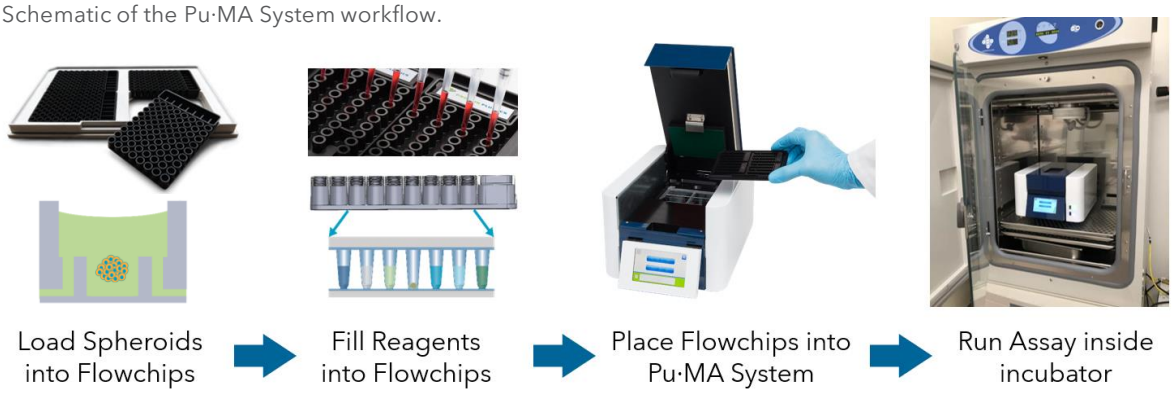
There is an increasing interest in using three-dimensional (3D) cell structures for modeling tumors, organs, and tissue to accelerate translation research[1]. Significant progress has been made in formation of such structures to recapitulate the *in vivo* environment but performing complex assays with them can be challenging. For example, manual treatment, staining, and processing of spheroids and organoids is typically labor-intensive and prone to disruption or loss of samples. Here we report on use of our microfluidic-based Pu·MA® System to perform automated assays with single spheroids followed by metabolite sampling using a novel in situ lysing technique. Metabolomic profiles of spheroids with and without treatment of compounds were obtained showing significant differences in their profiles.

Pu·MA System Workflow

Pu·MA System Flowchips are designed with chambers and reservoirs arranged in a convenient multi-well plate format (384-well spacings) and provide up to 32 tests per plate. Once spheroids and reagents are loaded into the flowchips, the plate is placed into the Pu·MA System and reagent exchanges are done automatically through microfluidic channels connected to the protected sample chamber (Fig 1). Multiple reagent exchanges can be performed enabling complex assay protocols to be run.

Assay programs are pre-loaded into the system and run using an intuitive touch-screen interface. The whole Pu·MA System can be placed in an incubator to run assays at 37°C and 5% CO_2. The system architecture and use of pneumatics to move fluids provides gas exchange to the sample chambers.

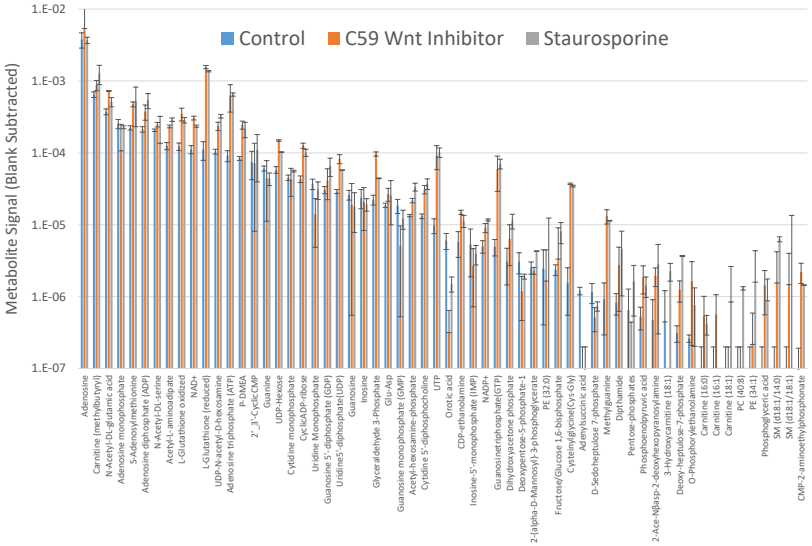
Figure 1. Schematic of the Pu·MA System workflow.



Single Spheroid Metabolomics

Use of Pu·MA System for automated media and compound treatment along with *in situ* lysing of spheroids for metabolomic analysis was successfully demonstrated. The ability to lyse organoids *in situ* in order to capture metabolomic profiles with minimal perturbation of the spheroids shows great promise for oncology research[2].

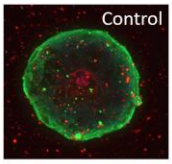
Figure 2. Metabolite signals from HCT116 spheroids.
Error bars represent +/- 1 SD (n=3)

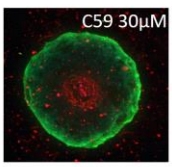


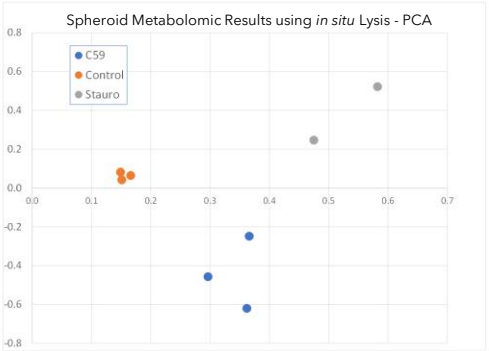
Assay Procedure

Colon cancer spheroids, HCT116 (spheroids with ~2K cells) were incubated at 37°C in the Pu·MA System Flowchips with compounds (C59 WNT inhibitor[3] (60 µM) and Staurosporine kinase inhibitor (1 µM)) for 2 hours and then automatically lysed by the Pu·MA System using an 80% acetonitrile solution. The cell lysate samples were collected and analyzed by mass spectrometry at the CRI Metabolomics Facility at UT Southwestern using un-targeted metabolomic analysis. Over 50 metabolites were measured in the lysates with excellent signal-to-noise. Significant differences in metabolomic profiles were observed between untreated (Control) and treated spheroids (Fig 2 and Fig 3).

Figure 3. Confocal images of representative spheroids and Principle Component Analysis (PCA) of results.







Cluster Analysis

Metabolomic results from the HCT116 spheroids were analyzed using hierarchical clustering (1 minus spearman rank correlation, complete linkage method). All signals were blank subtracted (Blank = well with no cells) prior to analysis. Cluster analysis showed excellent results with significant differences in metabolomic profiles between untreated and treated spheroids (Fig 4).

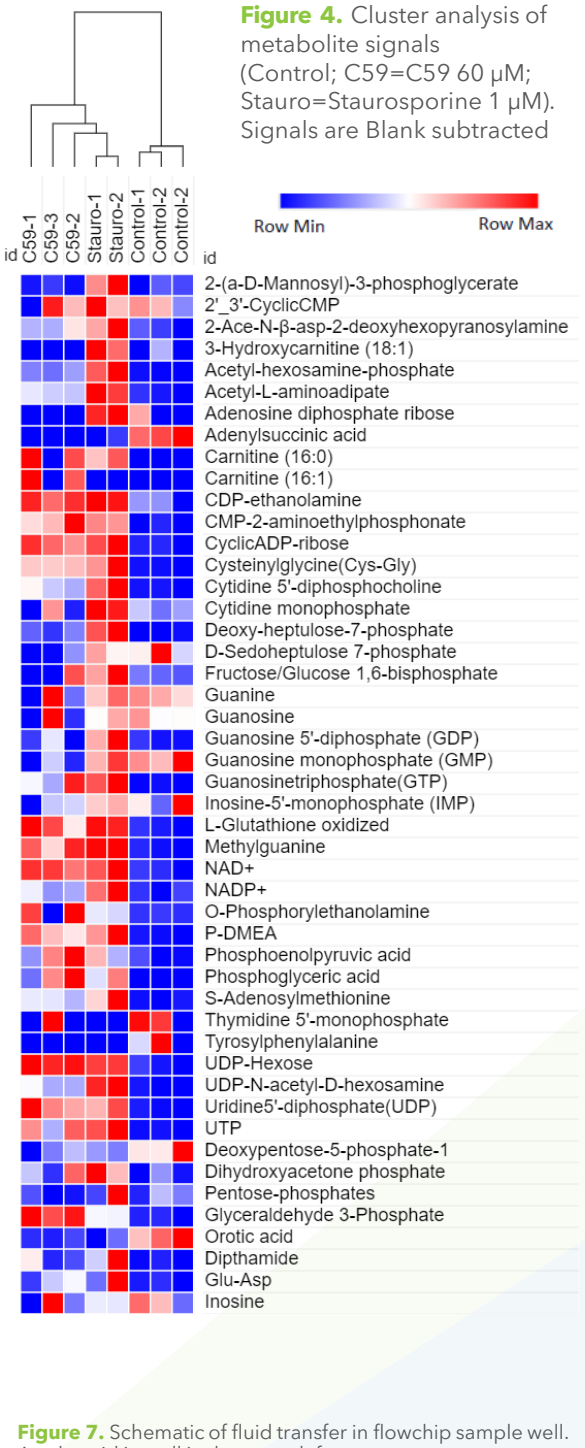


Figure 4. Cluster analysis of metabolite signals (Control; C59=C59 60 µM; Stauro=Staurosporine 1 µM). Signals are Blank subtracted

Conclusions

- We have demonstrated capabilities of a novel automated 3D Cell-based assay system that performs complex protocols with spheroids in an incubator environment.
- The Pu·MA System has been shown to perform *in situ* lysing of spheroids to provide highly sensitive metabolomic profiling with minimal perturbation.
- The ability to analyze spheroids and organoids *in situ* in order to capture toxicity information and metabolomic profiles shows great promise for oncology research.

Pu·MA System Microfluidics

The Pu·MA System can automatically perform complex assay steps using proprietary microfluidics. All reagents are loaded into flowchips and then incubation, media exchanges, cell secretion sampling and other steps are executed by the system program (Fig 5).

- 3D Cell models remain in the protected in bottom chamber
- Can exchange up to 95% of media without drying cells
- No direct fluid flow over cells to disrupt cell structures

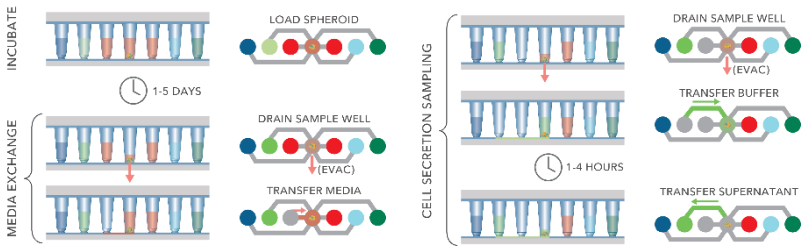


Figure 5. Schematic showing typical protocol steps available with the Pu·MA System. All steps are performed automatically inside an incubator.

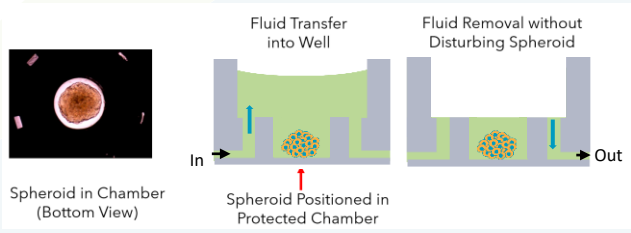
Repeatability

Fluid transfer repeatability was demonstrated by exchange of multiple buffers back and forth between Pu·MA System flowchip wells (Fig 6).

- 20 µl fluid transfers in 8 lanes, 3 repeats
- Fluid transferred to Well 3, incubated for 30 min, and then transferred back to origin well.
- Volume in origin well was measured gravimetrically
- Final Volume CV = 3%

Figure 6. Fluid transfer demonstration results. Error bars represent +/- 1 SD (n=8)

Figure 7. Schematic of fluid transfer in flowchip sample well. A spheroid in well is shown on left.



References
[1]Three-Dimensional in Vitro Cell Culture Models in Drug Discovery and Drug Repositioning, Langhans, S.A. Frontiers in Pharma. 2018, 9, 1.
[2]Metabolomics and Metabolic Diseases: Where Do We Stand? Newgard, C.B. Cell Metabolism 2017, 25, 4380.
[3]Cancer cell specific inhibition of WNT/β-catenin signaling by forced intracellular acidification, Melnki, S. et al., Cell Discovery, 2018, 4, 37

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