Pu·MA System® 3D for Spheroid Toxicity Testing and *In Situ* Imaging

Introduction

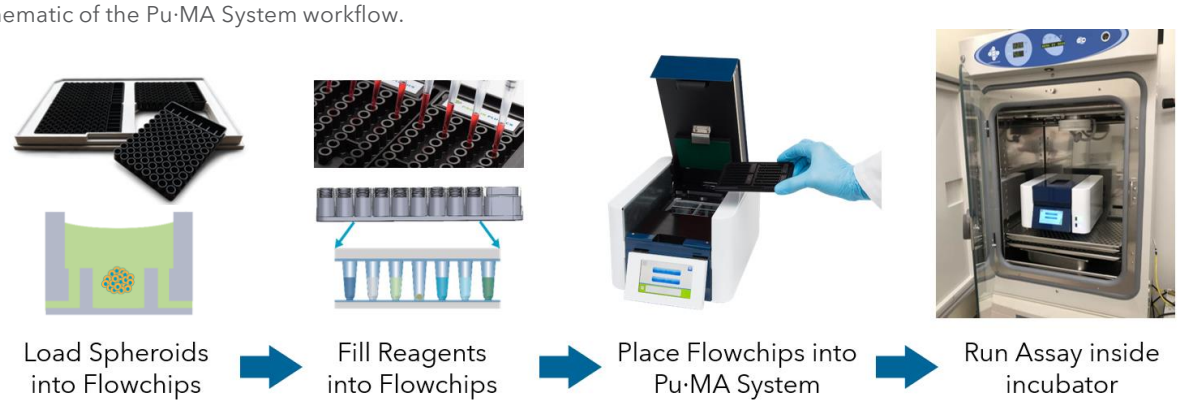
There is an increasing interest in using three-dimensional (3D) cell structures for modeling tumors, organs, and tissue to accelerate translation research[1]. Significant progress has been made in formation of such structures to recapitulate the *in vivo* environment but performing complex assays with 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 toxicity assays with spheroids. Spheroids can be incubated with and without compounds for 1 to 5 days and then analyzed *in situ* by high content imaging. Other cell viability methods such as measurement of ATP are also feasible.

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.

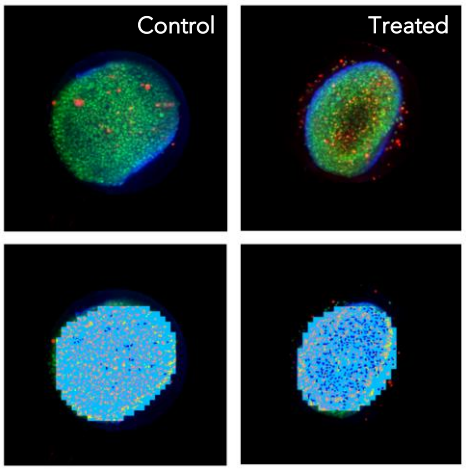
Figure 1. Schematic of the Pu·MA System workflow.



Spheroid Toxicity & Imaging

Capabilities of the system were demonstrated by assaying cellular toxicity over a 24-hour period with HCT116 spheroids. The spheroids were incubated in media with and without compound. The flowchips have a clear imaging window at the bottom of the sample chamber to enable high resolution imaging of the cell structures.

Figure 2. HCT116 Spheroids incubated for 24 hrs with and without 100nM Staurosporine. Cells were stained with a mixture of DAPI, Calcein AM and EthD. Images are 2D projections of a confocal image stack acquired on an ImageXpress Micro Confocal imaging system.



Assay Procedure

- Colon cancer cells (HCT116) cells (~2K cells) were grown and incubated for 48 hours until they formed tight spheroids.[2]
- The spheroids were transferred to a Pu·MA System flowchip in either media (Control) or media + compound (Treated) and placed into a Pu·MA System in an incubator.
- After compound incubation, spheroids were stained and washed prior to imaging.
- Imaging was done with an ImageXpress Micro Confocal Imaging System and analysis performed using the Custom Module Editor with MetaXpress Automated Imaging Acquisition and Analysis Software.

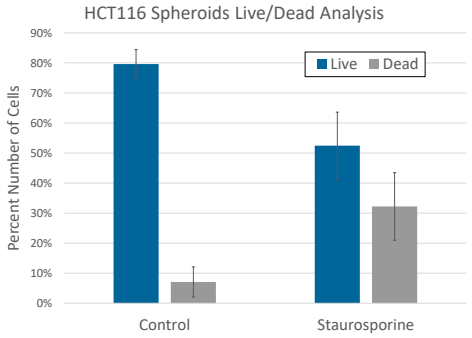


Figure 3. Live-Dead Analysis of HCT116 Spheroids incubated for 24 hrs with and without 100 nM Staurosporine

Cell Viability Assay

Cell viability was measured for HCT116 spheroids incubated with compounds for 48 hours in Pu·MA System flowchips. (Compounds: C59 30 µM; Mitomycin 30 µM; Etoposide 100 µM). Amount of ATP present in each spheroid sample was measured using the CellTiter-Glo assay (Promega). 10 µl of CellTiter-Glo reagent was automatically transferred into the sample wells and incubated for 10 min. Luminescence signal was measured using a SpectraMax iD5 Plate Reader (Molecular Devices) (Fig 4).

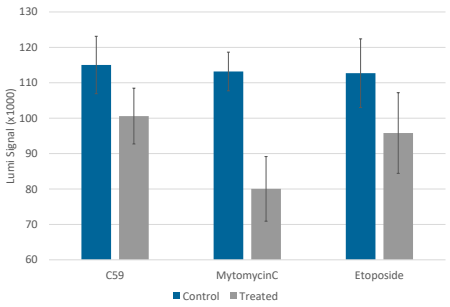


Figure 4. Relative amount of ATP in Treated and untreated (Control) HCT116 spheroids as determined by luminescence signal. Error bars = +/- 1 Std Dev (n=3)

Conclusions

- We have demonstrated capabilities of a novel automated 3D Cell-based assay system that performs complex protocols with spheroids in an incubator environment.
- Long term toxicity was assayed using high resolution confocal imaging of spheroids incubated with various compounds and Live/Dead staining and analysis profiling with minimal perturbation.
- The ability to analyze and image spheroids *in situ* in order to capture toxicity information shows great promise for oncology and toxicity research.

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]High-Content Assays for Characterizing the Viability and Morphology of 3D Cancer Spheroid Cultures, Sirenko, O. et al. Assay and Drug Dev Tech. 2015, 13, 402.

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 and 6).

- 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.

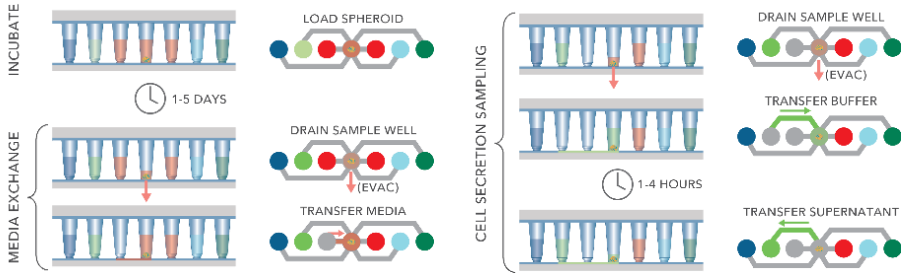
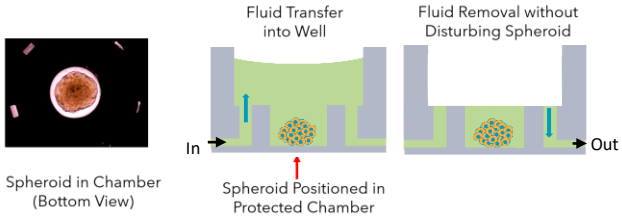


Figure 6. Schematic of fluid transfer in flowchip sample well. A spheroid in well is shown on left.

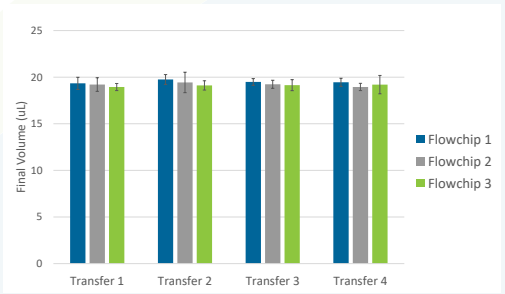


Repeatability

Fluid transfer repeatability was demonstrated by exchange of multiple buffers back and forth between Pu·MA System flowchip wells (Fig 7).

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



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