Dynamic multifunctional profiling of 3D cell models with an automated microfluidic-based assay system

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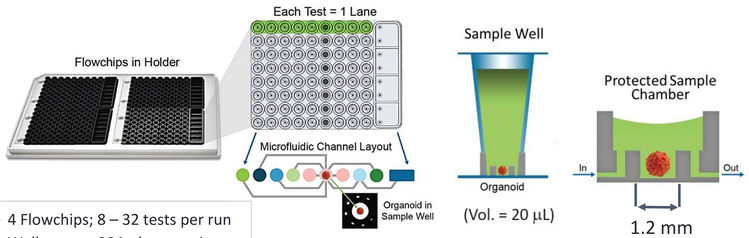
INTRODUCTION

3D cell models derived from patient tumors are highly translational tools that can recapitulate the complex genetic and molecular compositions of solid cancers and accelerate identification of drug targets and drug testing. However, the complexity of performing assays with such models remains a hurdle for their wider adoption. We present here results of disease modeling using primary tumor-derived 3D cell models with an automated organoid assay system (Pu·MA System). It uses a novel microfluidic flowchip that enables 3D high-content imaging (HCI), in situ supernatant sampling, and sensitive luminescence-based metabolite assays. In the example presented, tumoroids were formed from primary cells isolated from a patient-derived tumor explant, TU-BcX-4IC, that represents metaplastic breast cancer with a triple-negative breast cancer subtype. Assays were done using a Pu·MA System which performed media exchanges, compound treatments, and staining of tumoroids in a tissue culture incubator. Multifunctional profiling was done of tumoroids treated with anti-cancer drugs for 48 hours. Tumoroids were characterized by HCI using an ImageXpress Micro Confocal system with image analysis (MetaXpress software) to determine phenotypic responses. HCI was used to evaluate drug effects on cell viability and expression of E-cadherin and CD44. Supernatants were sampled from tumoroids at various timepoints and analyzed for metabolites using luminescence assays (Lactate-Glo and Glutamate-Glo). Lactate secretion was used to gauge tumoroid metabolism as a function of time and drug concentration. Complex dynamic behavior was observed for the different compounds that potentially can be used to understand patient-specific drug resistances. These methods can help provide an in depth understanding of drug sensitivity of individual tumor types, with important implications for the future development of personalized medicine.

Pu·MA SYSTEM TECHNOLOGY

Each Pu·MA System Flowchip contains eight lanes of reagent wells connected by microfluidic channels. Four flowchips are placed in holder that locates all wells in a 384 multiwell plate format providing for 32 samples per assay. The reagent wells in each lane hold media, compounds, or additional assay reagents. Tumoroids are placed into the sample well and located in a protected chamber at the bottom of the well. This allows reagents to be directed in and out of the Sample well without disturbing or drying out the microtissue **(Fig 1)**.



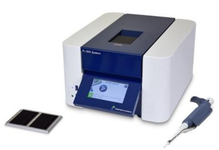
- 4 Flowchips; 8 – 32 tests per run
- Wells are on 384-plate spacings
- Thin optically clear COC bottom

Figure 1. Schematic of flowchips showing channel layout and sample well with proprietary protected chamber. The diameter of the sample well clear aperture is 1.2 mm.

INSTRUMENTATION

The Pu·MA System and 3D Flowchip features:

- Automated media exchanges occur with cells in protected chamber
- Supernatants can be collected to monitor cell secretion
- Spheroids can be imaged in the flowchip, or samples removed for immunoassay or metabolomics analysis
- Assay protocols can be edited via the Pu·MA System Software



ImageXpress Micro Confocal High-Content Imaging System includes:

- Five colors + transmitted light
- Environmental control
- Automated data analysis
- The system is controlled by MetaXpress High-Content Image Acquisition and Analysis Software

GloMax®-Multi+ Detection System with Instinct® Software

- Expandable multimode reader with dedicated optics for each mode
- Luminescence, Fluorescence, UV-Visible Absorbance

References

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MULTIFUNCTIONAL PROFILING

In order to understand the complex biology of tumors one needs to assess multiple aspects of tumor response to the treatment. The Pu·MA System enables multifunctional profiling (MFP) of individual tumoroids (**Fig 2**). Key aspects of the system includes:

- Compatibility with high content imaging
- Time-course sampling of supernatants
- In situ fixing and immunofluorescent staining

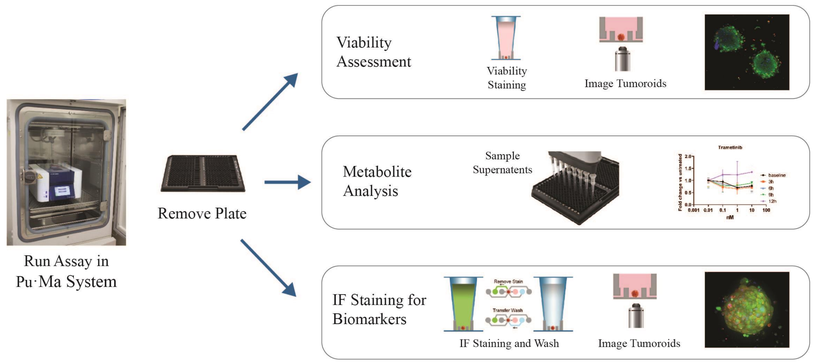


Figure 2. Multifunctional profiling workflow. Tumoroids are treated in Pu·MA System using automated protocols. Once treatment is finished tumoroids can be stained for viability markers and then imaged and analyzed for live or dead cells. In parallel, supernatant samples can be removed and analyzed for metabolites such as lactate and glutamate, or other secreted factors. Finally, tumoroids can be fixed and stained with antibodies against biomarkers such as E-cadherin and CD44.

TUMOROID FORMATION WORKFLOW

- Tumoroids were formed from TU-BcX-4IC cells derived from a primary tumor. The tumor exhibited rapid pre-operative growth despite combination neoadjuvant therapy with adriamycin, cyclophosphamide, and paclitaxel.
- 4IC cells were dispensed ~2,000 cells per tumoroid and incubated for 72 hours until they formed tight tumoroids.[2] The tumoroids were coated with magnetic nanoparticles[4] (NanoShuttle, Greiner Bio-One) to aid in the dispense and placement of the spheres in Pu·MA System flowchips **(Fig 3)**.

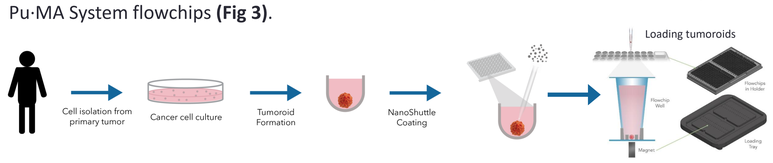
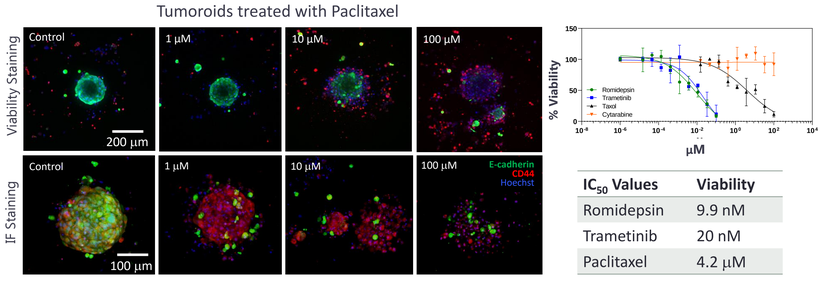


Figure 3. Workflow for tumoroid formation and loading into flowchips for multifunctional profiling. Tumoroids are labeled with magnetic nanoparticles (NanoShuttle) to aid in transfer and centering in the sample wells.

VIABILITY AND BIOMARKER RESPONSE

- 4IC tumoroids were treated for 24 hr with compounds from the NCI 60 panel of cancer therapeutics.
- First, tumoroids were stained with Hoechst, calcein AM, and EtHD-1 and imaged. Image stacks were analyzed for percent Live (calcein AM pos.) and Dead (EtHD-1 pos.) cells.[5,6]
- Next, tumoroids were fixed with 4% formaldehyde (Sigma) for 30min, washed (PBS), stained with FITC mouse anti-E-cadherin (BD Biosciences) and PE anti-human CD44 (BioLegend) antibodies overnight, and washed again.
- Tumoroids were then imaged on ImageXpress Micro Confocal system (**Fig 4**).



IC_{50} Values	Viability
Romidepsin	9.9 nM
Trametinib	20 nM
Paclitaxel	4.2 µM

Figure 4. Images of 4IC tumoroids treated with different concentrations of paclitaxel. **Top:** Viability-stained Live tumoroids imaged with 10X objective. **Bottom:** Immunofluorescence-stained Fixed tumoroids imaged with 20X WI objective. **Right:** Concentration response curves and 4- parameter fit results for PDOs treated with four compounds. IC_{50} values for romidepsin and trametinib were found to be within normal ranges while paclitaxel was significantly higher.[1,2] This is consistent with the response of the patient's tumor to treatment.

METABOLISM DYNAMICS

4IC tumoroids were treated overnight with compounds. Media + Compound was exchanged every 3 hours to provide a time-course sampling of metabolite secretions. Supernatants were analyzed for lactate using the Lactate-Glo assay kit (**Fig 5**). Supernatants were diluted 1:20 in media. 10 µL Samples + 10 µL assay reagents added to 384 multiwell plates. Plates were incubated for 60 minutes then luminescence read on GloMax plate reader

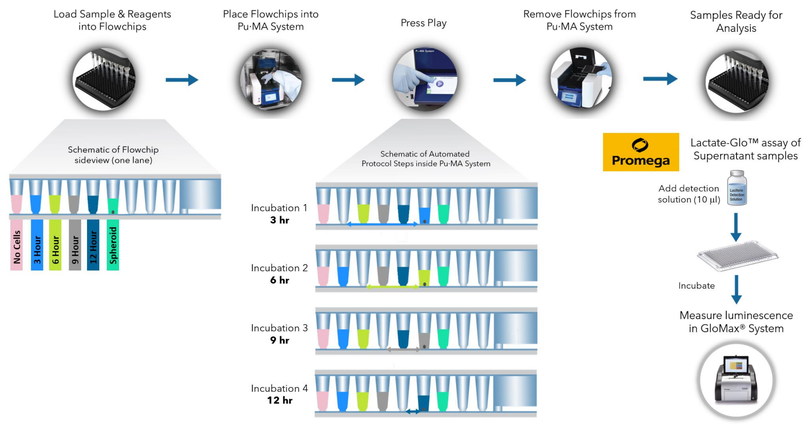


Figure 5. Workflow of metabolite secretion assays. Supernatants are sampled in situ by Pu·MA System, collected in the flowchips, then transferred into 384 well plates where they were analyzed for Lactate-Glo assay in a GloMax luminescence plate reader (Promega)

- Significant changes in lactate secretion were observed as a function of both time and concentration indicating a dynamic response of tumoroid glycolysis to treatment.
- A dynamic response of lactate secretion was observed (**Fig. 6**) indicating a change in metabolism of the tumoroids due to treatment. Further work is on-going to determine relation of metabolism dynamics to compound mechanisms of action (MOA).
  - Romidepsin – A histone deacetylase (HDAC) inhibitor
  - Trametinib – Selective reversible allosteric inhibitor of MEK1 and MEK2 activity
  - Paclitaxel – A mitotic inhibitor that interferes with microtubule growth

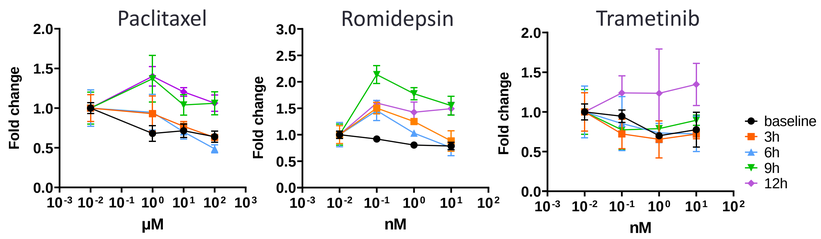


Figure 6. Relative amount of lactate measured in tumoroid supernatant samples as a function of compound concentration and incubation time. Data is shown as fold change relative to the baseline measurement (3 hr incubation with no compound). Each data point represents 3 independent tumoroids. Error bars = +/- 1 SDM

CONCLUSIONS

- We have demonstrated capabilities of a novel automated organoid assay system that performs complex protocols with 3D cell models in an incubator environment.
- Patient-derived organoids derived from primary tumors were assayed for compound response using high resolution confocal imaging of cell surface markers and viability stained for Live/Dead.
- The ability to analyze spheroids and organoids *in situ* in order to capture toxicity information and perform functional assays shows great promise for disease modeling.

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