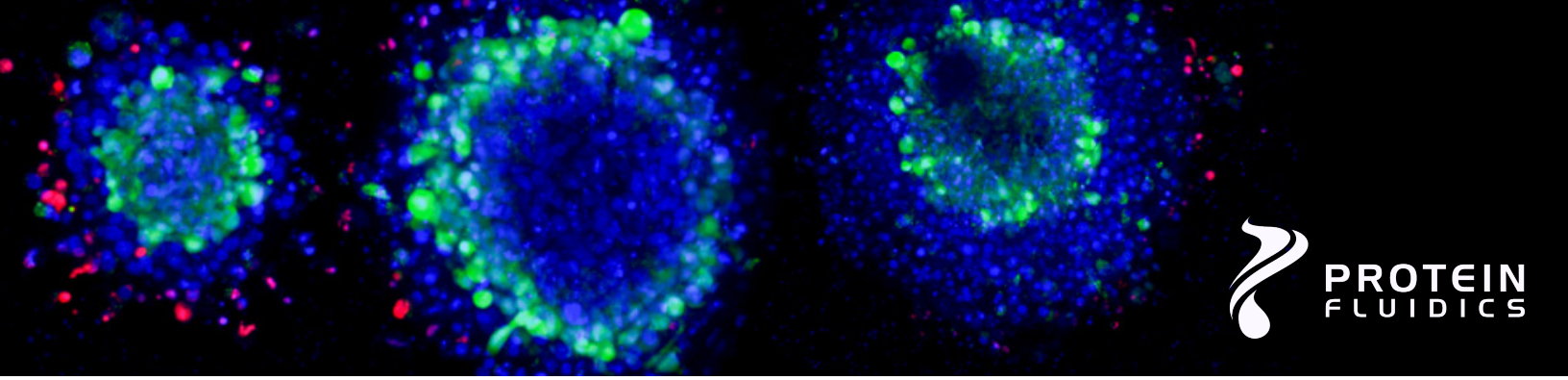


APPLICATION NOTE

Improved Assays with Magnetically Coated 3D Cell Models

Introduction

In recent years, the need to have physiologically accurate 3D cell models for research and drug development has been steadily growing. Researchers have been perfecting the formation and maintenance of various 3D models for understanding both disease and normal physiology [1,2]. Some of the limiting factors have been the ability to perform complex assays easily and quickly with these precious samples especially patient-derived material. Also, when performing drug treatments and assays in multi-well plates one is limited by the number of readouts per sample. The manual treatment, staining, and processing of spheroids and organoids is typically labor-intensive and prone to disruption or loss of samples. In addition, high content imaging can be problematic because organoids tend to locate at the edges of wells.

To overcome these issues during assays, we demonstrate the use of our Pu·MA System® 3D MAG and 3D Flowchips to perform automated assay steps with magnetically coated 3D cell models (Figure 1). NanoShuttle™ coated 3D cell models were transferred and centered into flowchip wells using magnets. The cell models were unaffected during the assays and easily imaged using a confocal microscope.

We demonstrate three different organoid assays that take advantage of the 3D Mag system:

- Media Sampling for secreted factors: Mouse islets were incubated with different glucose levels. Supernatants were collected *in situ* and analyzed for insulin secretion using ELISA.
- Drug treatment and staining: HeLa spheroids were treated with compounds for 24-48 hrs. Automated viability staining was used to determine concentration response curves.
- Immunofluorescence (IF) staining for biomarkers: TNBC patient-derived organoids[4] were assayed for drug response. PDOs were stained with IF markers and imaged in the flowchips.

Pu·MA System 3D MAG Workflow

Pu·MA System and 3D flowchips have been designed for streamlined automated organoid assays. Pu·MA System 3D MAG consists of the Pu·MA System with the 3D MAG modification which enables magnetically coated 3D cell models to be held within the protected sample chamber during the assay and subsequent imaging (Figure 1 & 2). The NanoShuttle treatment of formed organoids was done as per protocols from Greiner Bio-One[3]. In this application note we demonstrate a novel use of this bioprinting process coupled to our automated assay system. The magnetized 3D cell models are transferred into the flowchips with the help of a loading tray which ensures centering of the organoids (Figure 2). This has been designed specifically for use with our flowchips. The flowchips are then placed into the system within the incubator. The system architecture of the Pu·MA System and use of pneumatics to move fluids provides gas exchange to the sample chambers within the incubator environment.

The flowchips are in a convenient multi-well plate format (384-well spacings SLAS/ANSI standard) which makes them amenable to multichannel or automated liquid dispense system. They have optically clear bottoms for imaging with any fluorescence or confocal imaging system (Figure 1).

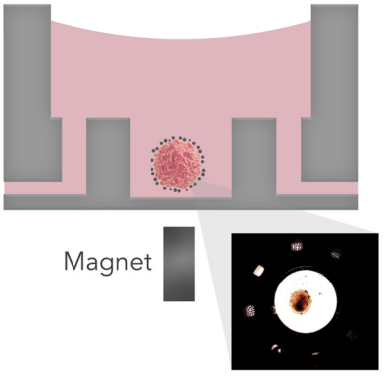


Figure 1. Schematic of a positioned spheroid within flowchip protected sample chamber in a Pu·MA System 3D MAG. Inset shows a bright field image of NanoShuttle coated mouse islets within the sample chamber

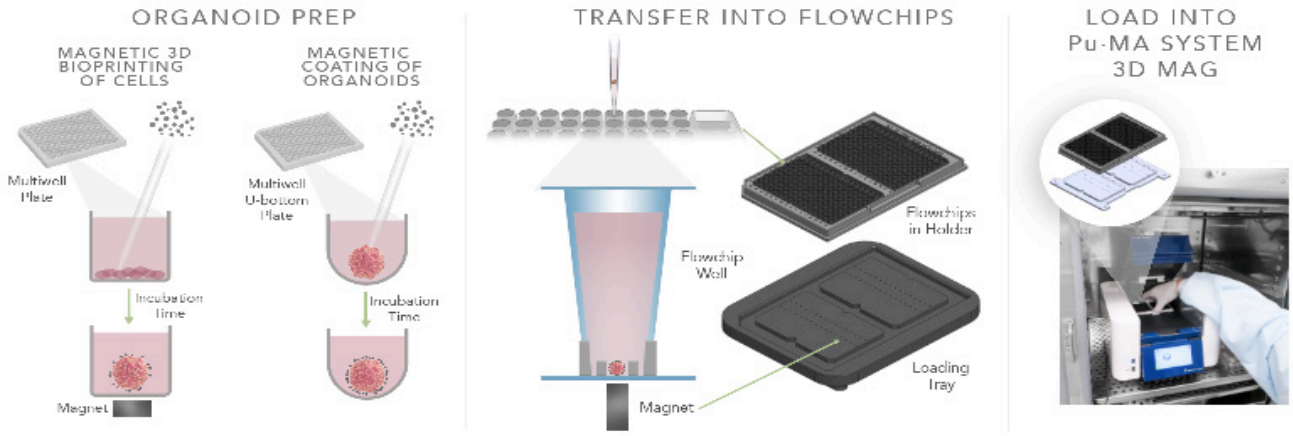


Figure 2. Assay steps for magnetic coating, transfer into flowchips and loading into Pu·MA System 3D MAG. The workflow used 1 µl of NanoShuttle (Greiner Bio-One) per sample to coat the formed 3D cell model. After the incubation they are transferred into flowchips with the aid of the loading tray. Reagents are loaded in the wells adjacent to the sample well and the flowchip holder placed into the Pu·MA System 3D MAG within the incubator.

Media Sampling for Secreted Factors

This application of the Pu·MA System 3D MAG, used NanoShuttle coated mouse islets sequentially treated with two different glucose concentrations with an equilibration step in between in an automated assay. The islets were briefly removed from Pu·MA System and imaged within the flowchip at each concentration (Figure 3A). Media was automatically collected from each concentration to measure insulin secretion by ELISA (R&D Systems) in response to the glucose levels. The islets were lysed for normalization. The islet data showed good correlation between secreted insulin in response to glucose concentration. (Figure 3B)

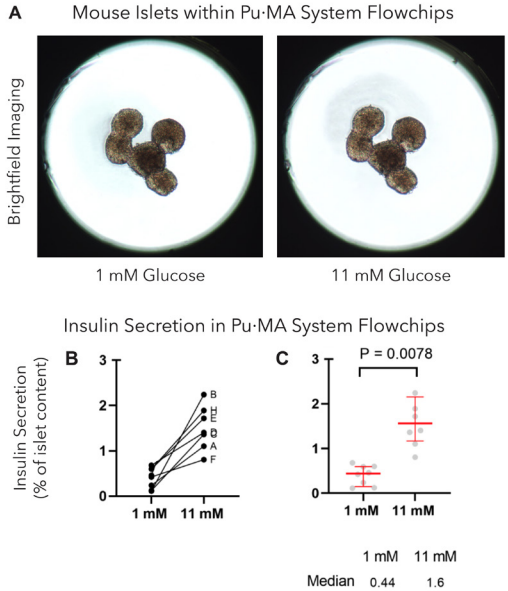


Figure 3. Media Sampling A Brightfield images of islets during the assay at 1 mM and 11 mM glucose concentrations. B Insulin secretion into the media at different glucose concentrations as measured by ELISA.

Drug Treatment and Staining

A second application of the Pu·MA System 3D MAG was demonstrated with HeLa spheroids for cell viability and cell death in response to the effects of selected cytotoxic drug treatments5.

HeLa spheroids were individually treated with different staurosporine concentrations (0 to 10 µM) for 22 hours, stained for 2 hours (Calcein AM, Hoechst and Ethidium Homodimer 1) and then imaged within the flowchips using ImageXpress® Micro Confocal Imaging System (Molecular Devices). Images acquired and analyzed as shown in Figure 4. Numbers of total, live or dead cells upon treatment with staurosporine were plotted from the image analysis data.

IF Staining for biomarker detection

PDOs were formed from TU-BcX-4IC (cells derived from a primary triple negative breast tumor) grown into tight spheres as described previously4 within 384-well ULA round bottom plates (Corning) and coated with NanoShuttle before transferring into flowchips. Automated drug treatments and immunofluorescence staining were performed within Pu·MA System 3D MAG.

The drugs for this study were Romidepsin and Trametinib treated at different concentrations for 48 hours and then stained for cell viability markers, imaged and concentration response curves analyzed (Figure 5A, 5B).

IF staining of PDOs were performed within the Pu·MA System following Romidepsin treatment. E-Cadherin and CD44 markers were detected in the PDOs after treatment, imaged using ImageXpress Micro Confocal Imaging System (Figure 5C). The results showed disruption of the PDOs upon drug treatments.

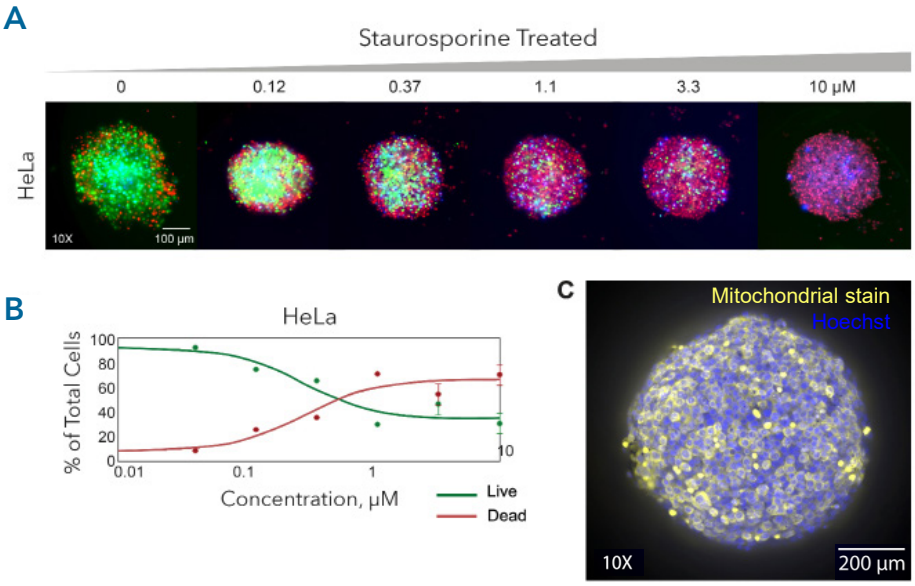


Figure 4. Drug treatment and staining A Confocal imaging of HeLa spheroids treated with increasing concentrations of Staurosporine (0 to 10 µM) and stained with nuclear stain (Hoechst, Blue), cell viability dye (Calcein AM, Green), cell death stain (EthD-1, Red). Images were taken using with a 10X Plan Fluor objective. B Graph indicating percentage cell number live versus dead cells upon treatment different Staurosporine concentrations (0.01 µM - 10 µM). C Untreated HeLa Spheroid stained and imaged within flowchips (Hoechst and Mitochondrial marker for cell viability (Mitotracker™ Orange).

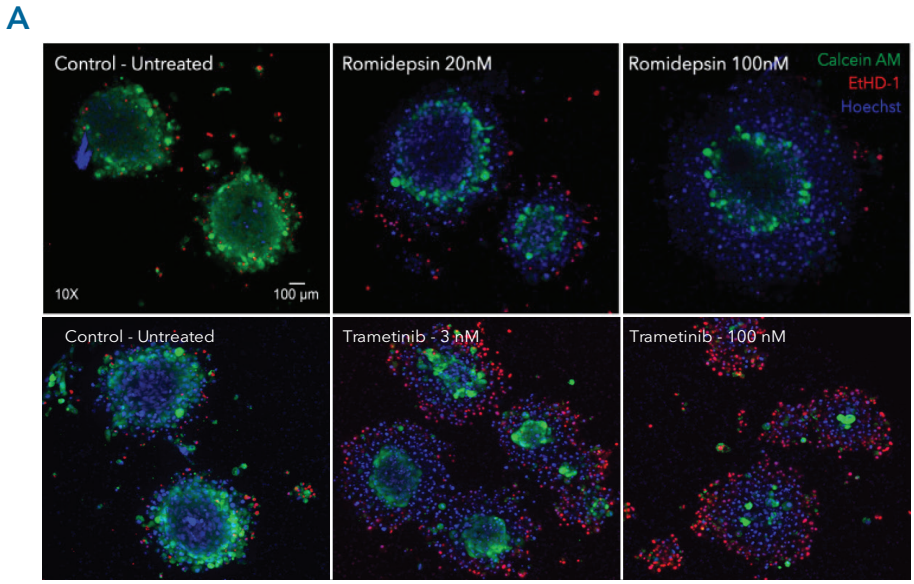
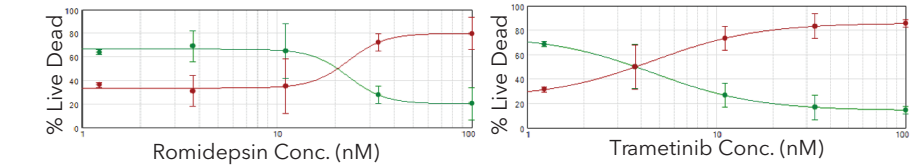
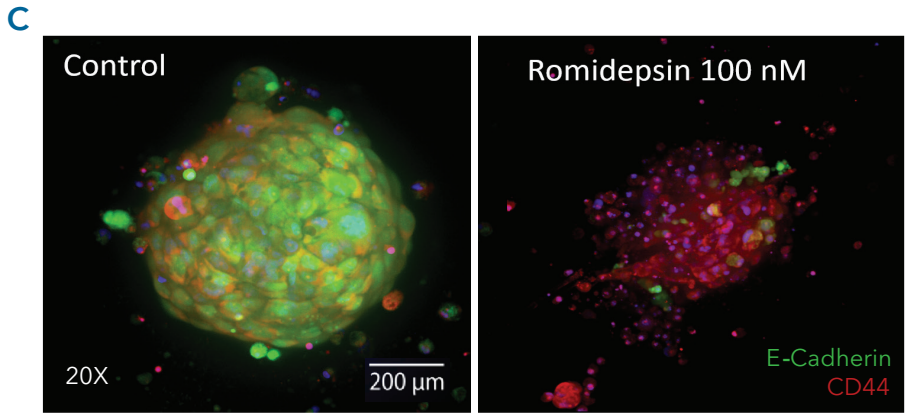


Figure 5. PDOs drug treatment assay. A Confocal Images of PDOs treated with romidepsin (Top) and trametinib (Bottom) and stained with nuclear stain (Hoechst, Blue), cell viability dye (Calcein AM, Green), cell death stain (EthD-1, Red). Images are maximum projections of confocal stacks acquired with a 10X objective.



B Concentration response curves for PDOs treated with romidepsin and trametinib. EC50 values for romidepsin and trametinib were found to be within normal ranges).



C PDOs labeled for E-Cadherin (Green) and CD44 (Red). Images are Maximum Projection of confocal stacks taken with 20X water immersion (WI) objective. Treatment with Romidepsin results in loss of E-Cadherin and disruption of organoids.

Summary

In this application note we have demonstrated improved assay capabilities of the Pu·MA System 3D MAG using magnetic coated 3D cell models. The combined system enhances the ability to perform:

- Automated organoid / spheroid processing
- *In situ* media sampling for secreted factors
- Drug treatment and multiparametric analysis
- Immunofluorescence staining for biomarkers

Complex assay protocols were performed using magnetic coated 3D cell models without disturbing the samples. We showed that we can quantify phenotypic changes such as cell death or secreted factors within physiologically relevant conditions and minimum perturbation. The ability to analyze organoids *in situ* to capture physiological information and perform functional assays shows great promise for disease modeling.

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Acknowledgements

This work was done in collaboration with Dr. David Piston and Dr. Alessandro Ustione (Washington University, St. Louis), Dr. Matthew Burow and Dr. Margarite Matossian (Tulane University), Dr. Oksana Sirenko and Matthew Hammer (Molecular Devices, Inc). NanoShuttle was kindly provided by Dr. Glauco Souza and Greiner Bio-One.

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 (schematic of fluid transfers is shown in Figure 6).

The key features are:

- 3D cell models remain in the protected sample chamber during the assay
- 95% of media can be exchanged without drying cells
- There is no direct fluid flow over cells to disrupt the cell structures

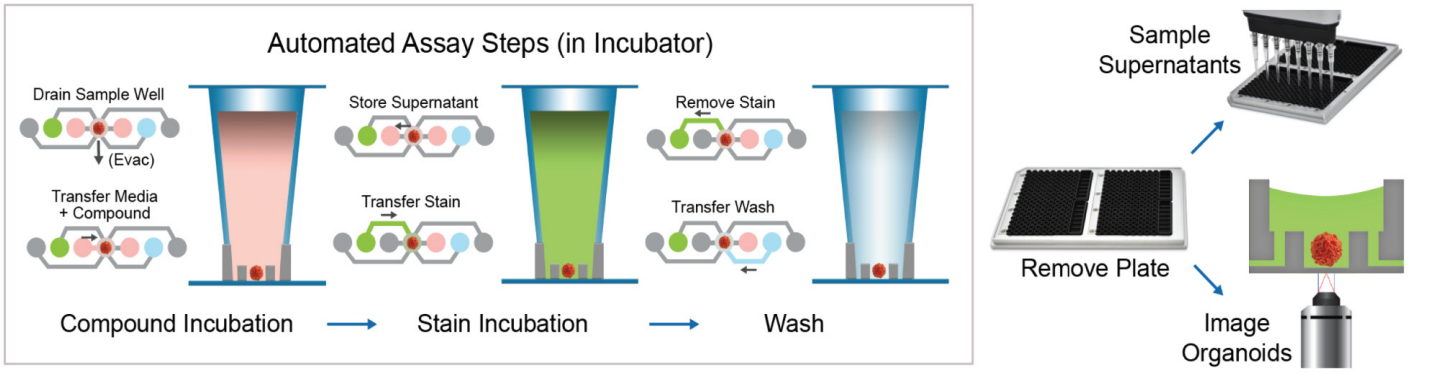

Figure 6. Pu·MA System microfluidics for automated assay steps for compound incubation followed by *in situ* supernatant sampling, staining and imaging

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