Automated 3D Co-Culture Assays for Immuno-Oncology Applications using Microfluidic Pu·MA System

Booth #903

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INTRODUCTION

Understanding the tumor - immune cell microenvironment provides insight into the complex interactions within the tumor between different cell populations and how to effectively target these interactions for treatment. With advances in immunotherapy there is a need for complex 3D tumor models because they more accurately represent the complexity and diversity of tumor microenvironments and cancer-immune cell interactions which influences the outcome of anti-cancer therapies. The increased complexities of 3D models add new challenges for 3D assay and advanced co-culture assay systems development.

We present an approach to perform **3D co-culture assays** using **automated microfluidic flowchips and Pu·MA System**. In this study MCF7 breast cancer spheroids were co-cultured with T-cells from human PBMCs. The assay format presented combines moderately high level of biological complexity and a more predictable behavior with simpler reproducible protocols.

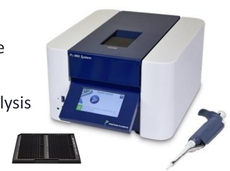
The platform allows the integration of:

- **3D cell model with delivery of immune cells + media exchanges**
- **Drug delivery**
- **Imaging capability to access phenotypic features and viability of the co-culture system**
- **Secretory activity profiling using biochemical-based readouts.**

INSTRUMENTATION

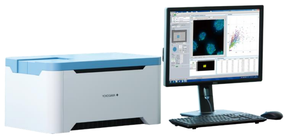
The Pu·MA System and flowchips

- Protected Sample Chamber technology prevents sample loss and damage
- Scheduled automated media exchanges to support 3D culture
- Scheduled automated collection of supernatants for secreted factors analysis
- Automated workflow solutions for IF Staining and other 3D cell assays
- In-Flowchip imaging



The CellVoyager CQ1 Benchtop High-Content Analysis System

- Confocal spinning disc technology
- High precision stage incubator and low phototoxicity
- Four fluorescence channels + transmitted light
- Integration with CellPathfinder high content analysis software



MICROFLUIDIC FLOWCHIP

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. Each test lane is designated to one organoids/spheroid sample and consists of a sample well connected to 8 reagent wells. Flowchip wells can be filled with any reagents (media, compounds, stains, etc.) depending on the assay configuration. Organoids/spheroids are loaded 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. The bottom of the flowchip is a thin cyclic olefin copolymer (COC) film which makes it compatible with high resolution imaging.

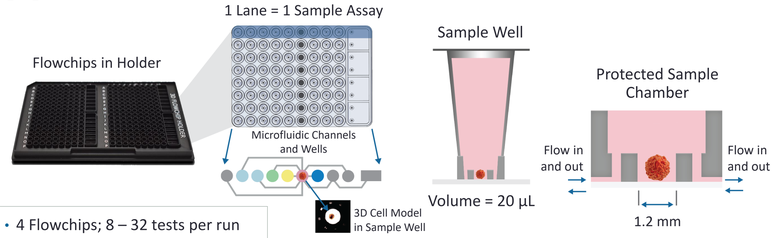


Figure 1. Schematic of Flowchips showing channel layout and sample well with proprietary protected sample chamber. The diameter of the sample well clear aperture is 1.2 mm and compatible with high-content imaging.

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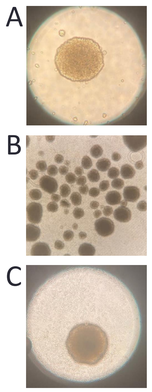
3D CO-CULTURE ASSAY WORKFLOW



Spheroid Formation

- MCF7 spheroids were formed from 5,000 cells in round bottom ULA 96-well plates over 72 hours and reached 300-400 µm size (Fig 2A).

PBMC Activation and T-cell Expansion

- PBMCs were activated by adding 25 µL ImmunoCult Human CD3/CD28 T-cell Activator (STEMCELL Technologies) per 1 X 10^6 cells.
- After activation, cells were expanded for 6 days in ImmunoCult-XF T-cell Expansion Medium supplemented with IL-2 (STEMCELL Technologies) (Fig 2B).

Loading Flowchip and Automated Assay

- Spheroid was loaded to the Sample well of the flowchip.
- T-cells were loaded into Reagent well of the flowchip and 5,000 cells were delivered directly to the Sample well via microfluidics channel (Fig 2C).
- Automated media exchanges were done by the Pu·MA System (Fig 2D).

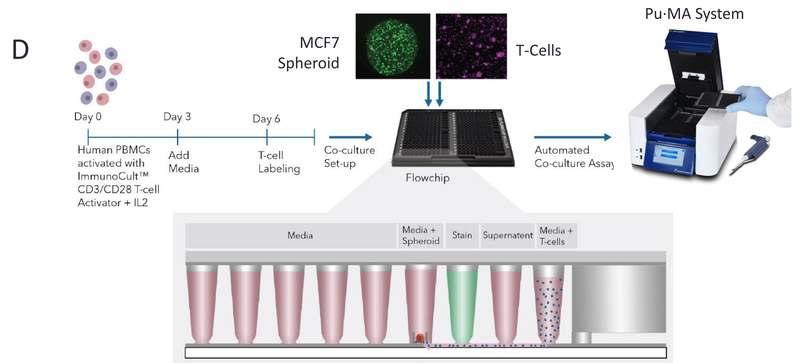


Figure 2. 3D co-culture workflow, Spheroid formation (A), PBMC activation and T-cell expansion (B), loading flowchip and automated assay workflow (C and D).

CONFOCAL IMAGING OF CO-CULTURE

- MCF7 spheroids were pre-labeled with CFSE (Carboxyfluorescein succinimidyl ester – non-toxic live cell staining dye).
- Activated T cells were pre-labeled with CellTracker Deep Red Dye (Thermofisher Scientific).
- Spheroids and activated T-cells were co-cultured in the flowchip for 48 hr.
- Confocal images were acquired in the flowchips using CellVoyager CQ1 High-Content Analysis System. A 150-200 µm z-stack of images with a 5 µm z-step was acquired using 488 and 561 nm channels.

Maximum intensity projection (MIP) images were acquired at 4 hours and 48 hours of co-culture, along with controls (either T-cells or the MCF7 spheroid alone, Fig 3). At the beginning of co-culture, the MCF7 spheroid (green) displayed a compact and round shape. The spheroid was surrounded by clusters of activated T-cells (magenta). Upon interaction of cancer spheroid and T-cells we observed T-cell redistribution and localization within the spheroid. The T-cell effector activity resulted in spheroid loss of shape and integrity with increased presence of damaged and dead cancer cells. The control spheroid (no T-cells exposure) maintained its shape, architecture, and remained intact (Fig 3).

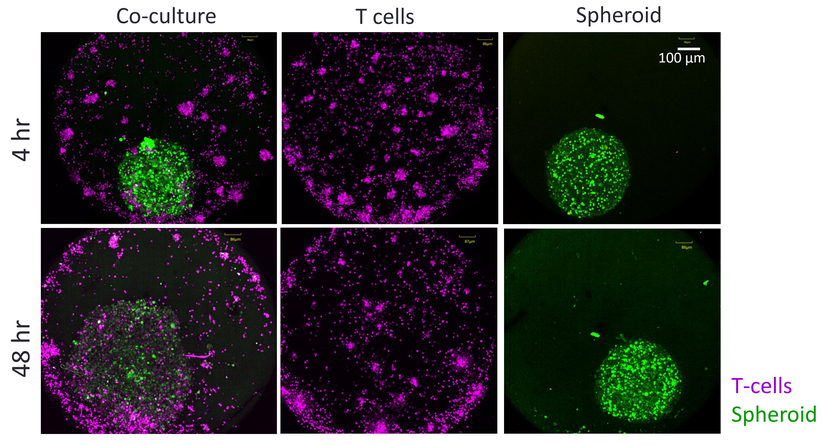


Figure 3. Confocal MIP images of T-cell – MCF7 spheroid interaction over 48 hours in co-culture.

AUTOMATED IF STAINING OF CO-CULTURE

- Co-cultures were fixed in the flowchip after 48 hours.
- Automated IF staining was performed within Pu·MA System at room temperature (Fig 4).
- Co-cultures were stained for CD3 (T-cell marker) and nuclei (Hoechst).

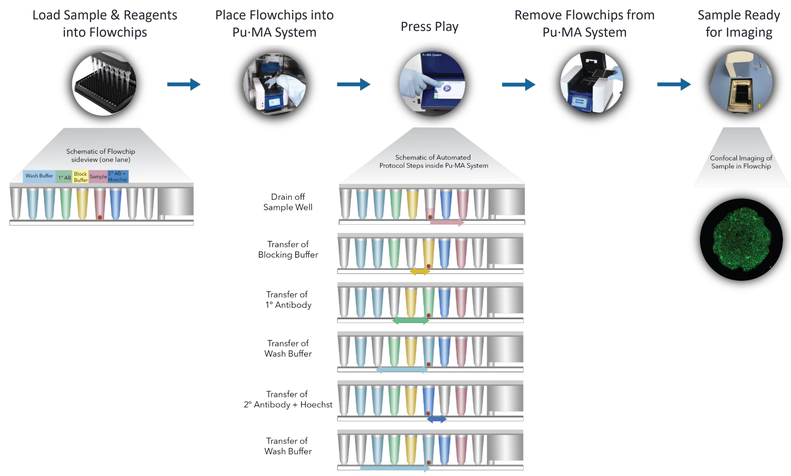
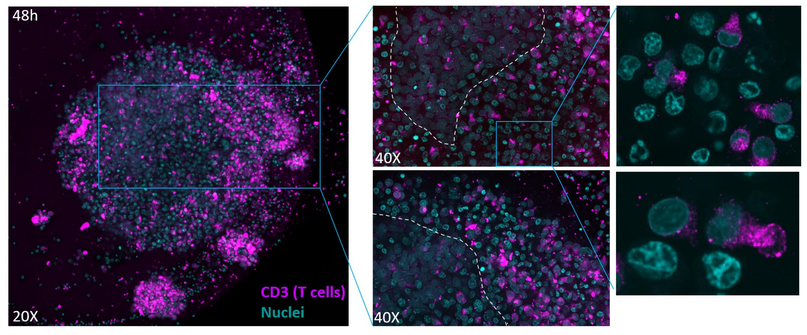


Figure 5. Schematic of automated IF staining protocol that was used to stain MCF7 spheroid – T-cell co-cultures

IMAGING OF STAINED CO-CULTURES

- Confocal images were acquired with a 20X long working distance and 40X objectives using the 405 nm (Nuclei Hoechst) and 488 nm (CD3) channels.
- A 120 µm z-stack of images separated by 10 µm was acquired (Fig 5).

IF staining demonstrated significantly more T-cells present at the edge of spheroid and they were associated with significant cancer cell damage (Fig 5). The area closer to the middle of spheroid showed presence of fewer T-cells and more intact spheroid architecture with healthier looking cells compared to peripheral areas.



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CONCLUSIONS

- In this study we demonstrated how immune-cancer cell co-culture assays can be streamlined using automated Pu·MA System.
- Automated immunofluorescence staining protocol, combined with high-content imaging, allows deeper studying the interplay between tumor microenvironment components and different tumor infiltrating immune cell populations, their spatial organization features.
- This is extremely beneficial towards the goal to identify new treatments and assess individual therapeutic approaches to advance personalized medicine.

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