Semi-automated, scaffold-free organoid culture workflow

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Introduction

T Cell-CRC PDO interaction assay

Among the therapies for treating cancer, immunotherapy—especially chimeric antigen receptor (CAR) T-cell therapy—is an effective emerging treatment paradigm for solid tumors. CAR T cells are genetically altered T cells that when injected back into patients, they help arm the immune system to target and destroy cancer cells. Though T-cell therapy has achieved much success with immunotherapy in treating blood cancers, it remains challenging in targeting solid tumors due to the intrinsic solid tumor microenvironment (TME) which suppresses the tumor-killing ability of T cells. Thus, TME plays an important role in the treatment of solid tumors and finding an approach of mimicking TME in vitro is essential for CAR-T cell screening. Studies show that 3-dimensional (3D) patient-derived organoids (PDO) model more of the physical and chemical cues present within the TME which traditional 2D cell line cultures lack. They also show similar responses to drugs as original tumors, suggesting the value of using PDOs to improve therapeutic outcomes.

Despite the benefits associated with the use of PDOs, there are significant barriers that hinder their widespread adoption in drug discovery, i.e., the costly and highly labor-intensive processes associated with their growth and maintenance. To address the challenges associated with the use of PDOs in large-scale applications, a semi-automated bioreactor was developed for high-throughput expansion of assay-ready organoids. Further, to free the burden of using Matrigel domes for organoid culture in assays and skip the dome solidification step, we developed a dome-free organoid workflow to quantify the efficacy of the cells in solid tumors, automatically seeding and culturing PDOs in a novel nano film coated R^{3}CE cell culture plate (AcroCyte Therapeutics) enabling spontaneous dome-free 3D organoid formation using the CellXpress.ai™ Automated Cell Culture System (Molecular Devices). Using bioreactor-expanded patient-derived colorectal cancer organoids (CRCs), activated human peripheral blood mononuclear cells (PBMCs) were added to CRCs in a 96-well plate and monitored every 4 hour for 4 days. We used Custom Module Editor (CME), an analysis module in IN Carta® Image Analysis Software (Molecular Devices) to segment the T cells and the organoids, measuring the penetration distance of each T cell within the organoid. We found that phorbol myristate acetate/ionomycin (PMA/i) activated T cells exhibit distinct penetration behavior known as chemotaxis with different concentrations of PMA. Thus, the CellXpress.ai system together with the image analysis workflow provides an innovative approach for in-vitro T-cell screening.

Methods

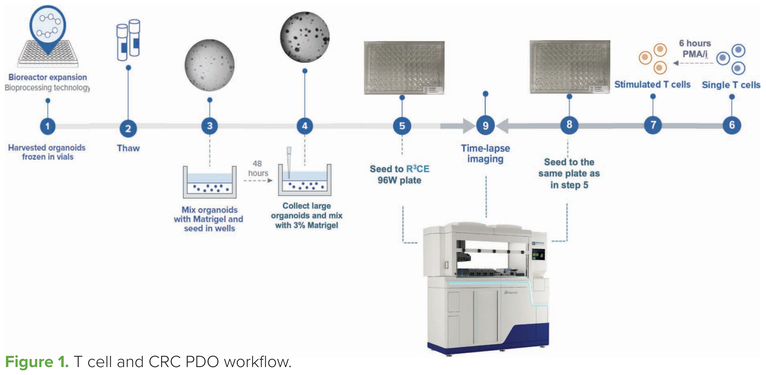


Figure 1. T cell and CRC PDO workflow.

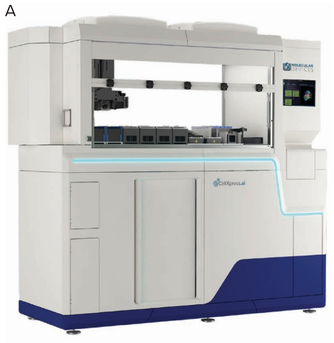
Bioreactor-expanded patient-derived CRCs were first mixed with 80% Matrigel and grew 48 hours before collection. The collected large organoids were then stained with MitoTracker Red and mixed with 3% Matrigel before seeding into 96-well plate using the CellXpress.ai system. The thawed PBMC/T cells were stimulated with PMA at 1ng/mL, 5ng/mL, 25ng/mL, 125ng/mL, or 625ng/mL combined with ionomycin at 1µg/mL for 6 hours and stained with CellTracker Green before being added to the same plate for co-culture using the CellXpress.ai system. Time-series images every 4 hours were acquired automatically with AI-powered imaging using 10X air objective and 2D projection.

Experiment setup

The CellXpress.ai Automated Cell Culture System

The CellXpress.ai Automated Cell Culture System automates the process of seeding, culturing and passaging organoids and monitoring by imaging, with pre-established workflows and fine-tuning settings. With the CellXpress.ai system, we automated organoid seeding and T-cell seeding and monitoring in three phases. We first used a pre-configured SEEDING workflow to plate the organoids mixed with 3% Matrigel into a R^{3}CE 96W plate in Phase 1, followed by a pre-configured COMPOUND ADDITION workflow to plate the T cells stimulated with different concentration of PMA into the same plate in Phase 2. The co-culture of CRC organoids and T cells were monitored every 4 hours for 4 days using a pre-configured IMAGING AND ANALYSIS workflow in Phase 3.

A



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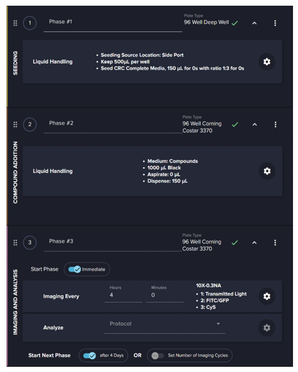

Figure 2. A. The CellXpress.ai Cell Culture System that integrates a liquid handler, an incubator with up to 154 plates, an imager with TL and up to 6 fluorescence channels and waste system; B. Three phases of Seeding from 96W deep well plate to 96W R^{3}CE plate , Compound Addition from 96W compound plate to 96W R^{3}CE plate and Imaging and Analysis workflow on the 96W R^{3}CE plate .

R^{3}CE plate

The R^{3}CE plate is a scaffold-free single-cell proliferation 3D cell culture platform, that is coated with novel nanofilm enabling culture of 3D tissues from a single cell and rare cells. To develop this scaffold-free workflow, we used R^{3}CE 96W plate to maintain the structure of the 3D organoids, allowing free movement of T cells around organoids.

Analysis

IN Carta analysis software with Custom Module Editor was used to analyze the images acquired from the CellXpress.ai system. The raw images of T cells were first preprocessed to smooth the image and remove the background, followed by the segmentation of T cells. The same procedure was performed on CRC organoid images to preprocess and segment the organoids, generating the masks of the organoids. The organoid masks were then used to generate a distance image where the intensity values at a point represent the distance of that point to the nearest white pixel in the organoid masks. Distance image was then overlayed with T cell masks to measure the T cell penetration distance into the organoids.

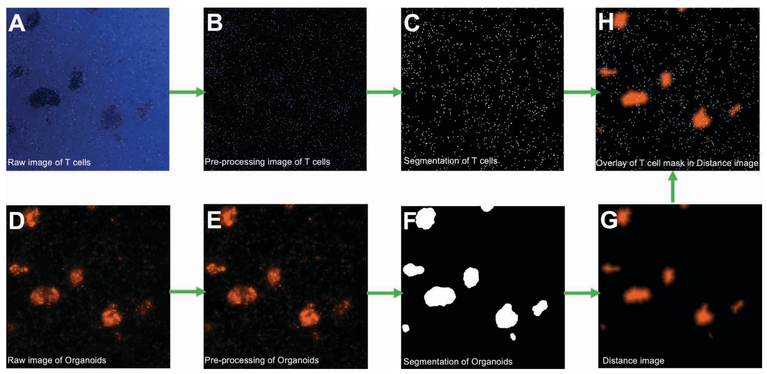

Figure 3. T cell penetration distance analysis workflow. A. Raw image of T cell; B. Pre-processed image of T cells; C. Masks of T cells; D. Raw image of CRC organoids; E. Pre-processed image of organoids; F. Masks of organoids; G. Distance image; F. Overlay of T cell masks in distance image.

Results

PMA dependent increase in T cell interaction with CRC organoids

PMA/i is known to enhance T cell mobility. We demonstrate this fact by the PMA concentration related T-cell infiltration or interaction pattern into the CRC organoids. Stimulated T cells clearly show more significant co-localization than unstimulated T cells (Figure 4, last column).

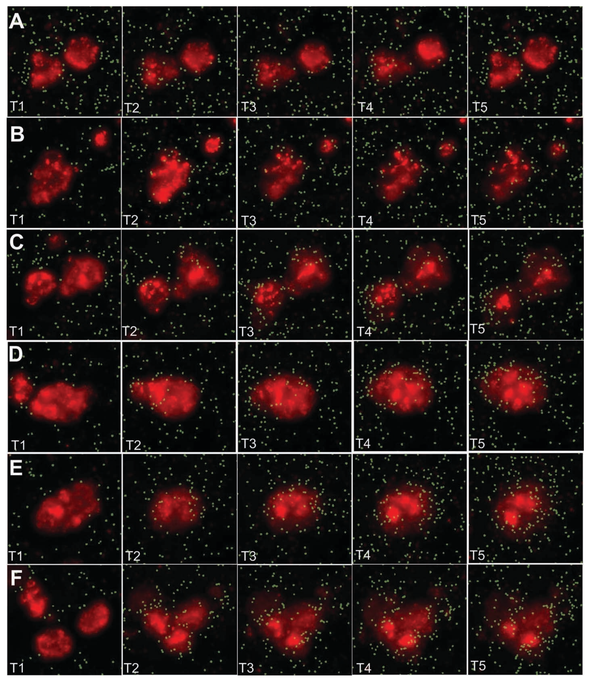

Figure 4. Representative overlays of organoid image and T cell masks for the five time points. A. Unstimulated T cells; B. T cells stimulated with 1ng/mL PMA; C. T cells stimulated with 5ng/mL; D. T cells stimulated with 25ng/mL; E. T cells stimulated with 125ng/mL; F. T cells stimulated with 625ng/mL.

T-cell interaction analysis

To quantify the effect of PMA of different concentrations on T cells, we calculated the total penetration distance of T cells inside the organoids $\frac{\Sigma_{i\in INSIDE^t} d_i}{\Sigma_{i\in INSIDE^t}+\Sigma_{i\in OUTSIDE^t}}$ and counted all the penetrated T cells, respectively, normalized by the total number of T cells to compensate the seeding variability and photobleaching. We found that 1ng/mL PMA has little effect on the T-cell penetration while higher concentrations have significant effects. Moreover, concentrations equal to or higher than 25ng/mL stimulate T cells more profoundly in the beginning then exhaust. In contrast, 5ng/mL PMA activates T cells energetically across the time.

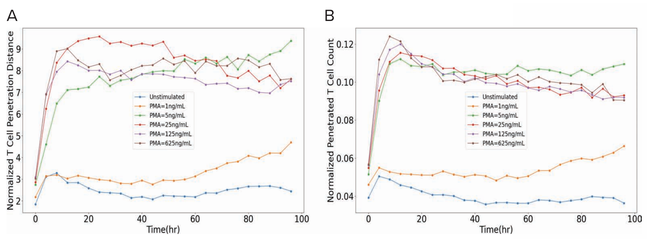

Figure 5. A. Normalized T-cell penetration distance; B. Normalized penetrated T cell count.

Conclusion

- We used the CellXpress.ai Automated Cell Culture System to automatically seed the organoids and T cells for co-culture and monitor every four hours.
- We used IN Carta image analysis software to analyze the T cell penetration into the organoids and quantify the PMA effects.
- We demonstrated the feasibility of using the CellXpress.ai system as an integrated solution for T-cell screening and in-vitro cell therapy.