T cell clone selection based on tumor co-cultures by TROVO System

Introduction

The Enrich TROVO platform has the ability to create hydrogel based microwells, image single T cell and cancer cell interactions, and retrieve T cells of interest for downstream analysis or expansion. One of the novelties of this platform is its ability to capture and retrieve cells. Using the data collected from imaging, the platform provides single cell kinetics, including killing rate, proliferation rate, and engaging time to predict in-vivo performances amongst different cell clones. This is used to robustly aid in determining microwells of interest for CAR-T development and TCR-T identification.

Microwell Coculture Setup

The Enrich TROVO platform has the ability to print 14,400 uniform microwells (MW) on a 6 well cell culture plate in just under 1 hour. Cancer cells and T cells are combined at a chosen E:T ratio and plated onto the microwells. The co-culture is imaged every few days with cancer cell rechallenging every 3 days. This allows you to track the T cells and cancer cells proliferation and interactions to determine which microwells will be captured (Fig.1).

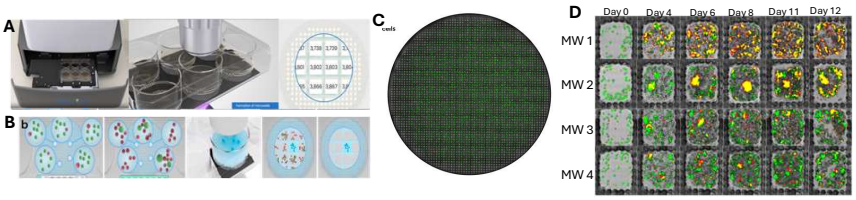

Figure 1. T cell/cancer cell co-culture over 12 days. T cells (red) and tumor cells (green) are plated on hydrogel microwells (MW) and imaged over a 12 day period.

Capturing and Retrieval Workflow

To capture, a biocompatible gel is added to the microwells and a T cell vs Cancer cell confluency graph is used to select microwells of interest. These selected microwells can be seen highlighted in red on the summary page (Fig. 2A) and in blue on the whole well image (Fig 2B). Once captured, the microwell is removed and the well washed to get rid of unwanted cells, leaving behind only the capture gels containing the cells of interest (Fig. 2C). Live T cells can be released enzymatically (Fig 2D) for downstream analysis such as sequencing (Fig 3), REP expansion, and tumor-killing verification (Fig.4).

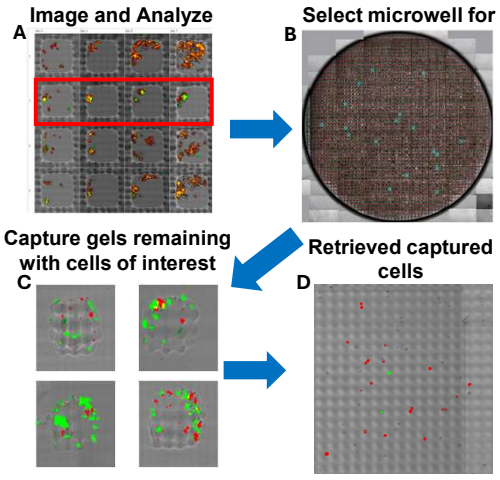

Figure 2. Capturing and retrieval workflow. Microwells of interest are captured by selecting a grouping of microwells from a T cell vs cancer cell graway,d captured by our biocompatible gel. The unwanted cells can be washed away and the capture gel can be enzymatically degraded to release the cells for downstream processing.

Application Highlights

1. T Cell Isolation from Solid Cancer

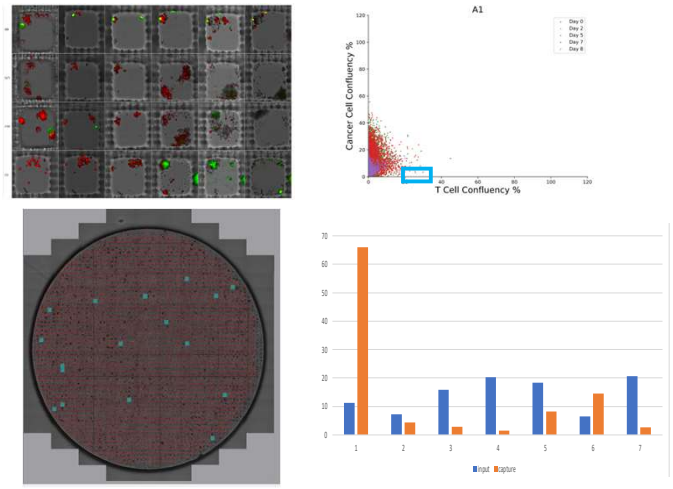

Figure 3. Isolating T cells from solid cancer. CAR-T cells and cancer cells were plated at an E:T of 3:10 and co-cultured for 8 day, imaging daily. Using the cancer cell vs T cell confluency graph microwells of interest were selected and T cells retrieved for NGS analysis.

2. T Cell Isolation for Expansion

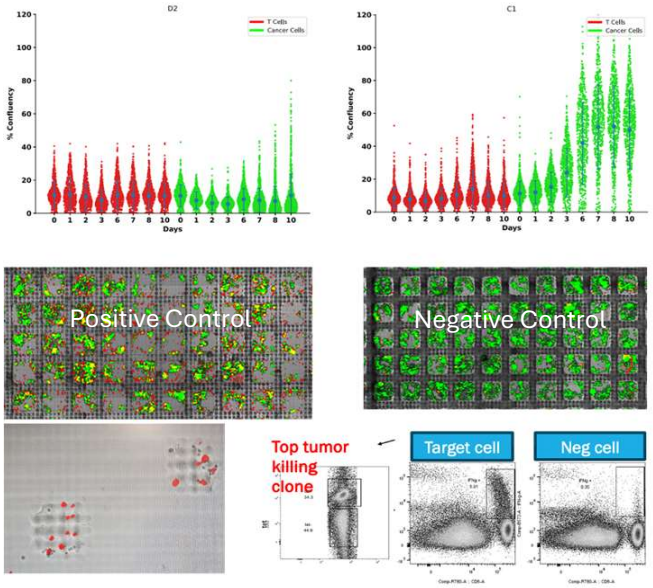

Figure 4. Capturing and expanding T cells. T cells of interest were captured using the TROVO platform and then expanded in culture. The cells maintained normal phenotypic expression and functionality.

Acknowledgment

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