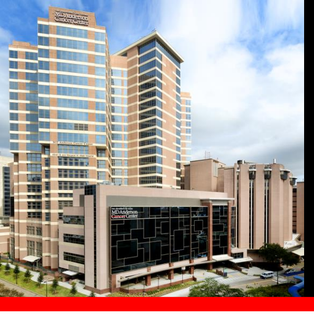

Development and validation of a high-throughput 3D cytotoxicity assay to predict CAR T cell efficacy in solid tumors

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Introduction

Chimeric antigen receptor (CAR) T cell therapy has been shown to be highly effective against leukemia and lymphoma. However, its efficacy in treating solid tumors has been limited due to several factors that include tumor antigen heterogeneity, immunosuppressive microenvironments, and nutritional constraints. Therefore, development of effective cellular therapies for solid tumors requires adjuvants that can help T cells overcome these intrinsic barriers. Rapid determination of these factors requires high-throughput screening of combinatorial libraries of cytokines and checkpoint inhibitors in a representative *in vitro* setting that can accurately predict CAR T cell function in solid tumors. Spheroids are three-dimensional (3D) cell cultures that can effectively mimic solid tumor microenvironments. To this end, we have utilized a rapid 3D bioprinting method to produce a large array of spheroids in 384-well plates to rapidly identify optimal conditions that maximize CAR T cell infiltration and cytotoxicity in a 3D setting.

Methods

We used magnetic 3D bioprinting to produce a large array of spheroids composed of U87 glioma cells expressing green fluorescent protein (GFP) in a 384-well plate. CAR T cells, labeled with Hoechst stain for fluorescent visualization, were added to the spheroid cultures in the presence of cytokines and checkpoint inhibitors to determine optimal combinations for T cell function. Time-lapse fluorescence microscopy was used to visualize and measure tumor spheroid viability (GFP intensity) and T cell infiltration by acquiring images over a 48-hour time period. Flow cytometry was also used prior to the addition of the CAR T cells and cytokines to assess for the presence of immunosuppression markers in the spheroid cells.

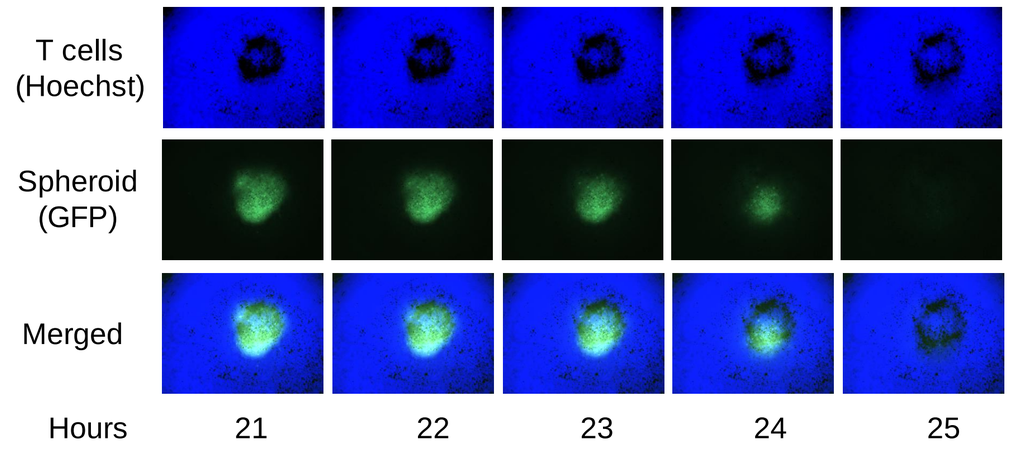

Figure 1. Fluorescent microscopy images of select timepoints after addition of CAR T cells to U87 spheroids. The top images depict Hoechst-stained CAR T cells as they infiltrate the U87 cell spheroid. Response of the spheroids to cytotoxic activity of CAR T cells is depicted by the decline in GFP fluorescence (middle row). Merged channel images (bottom row) illustrate coincidence of infiltration and tumor response. Each set of images is shown in sequential order at hourly intervals.

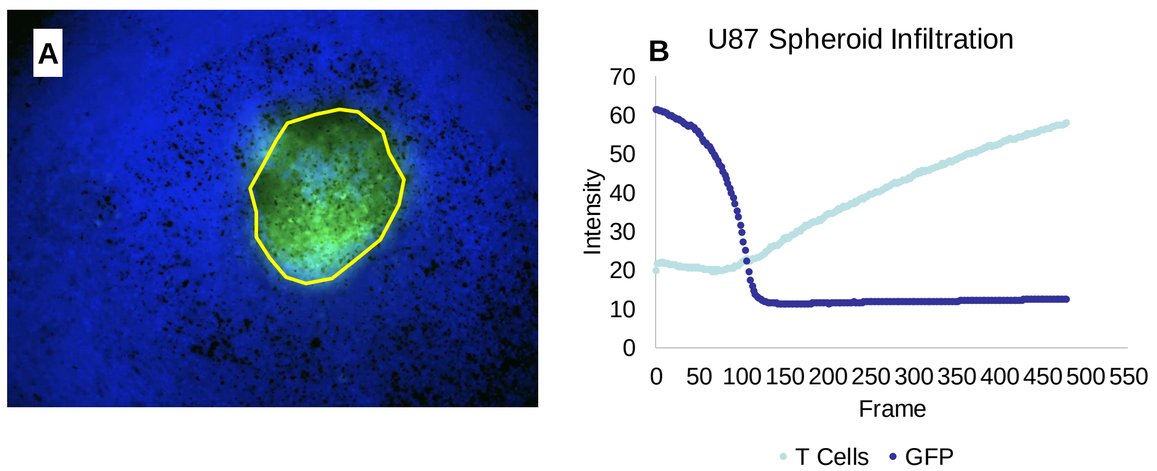

Figure 2. Time-dependent quantification of CAR T cell infiltration into U87 spheroids. A) The video was taken using fluorescence microscopy to track T cell infiltration (Blue Hoechst stain) into a U87 cell spheroid (GFP) to quantify infiltration over time within a region-of-interest (ROI; yellow outline) drawn around the perimeter of the spheroid. B) Fluorescence intensity measurements over time within the ROI show an inverse correlation between the CAR T cell infiltration and spheroid viability.

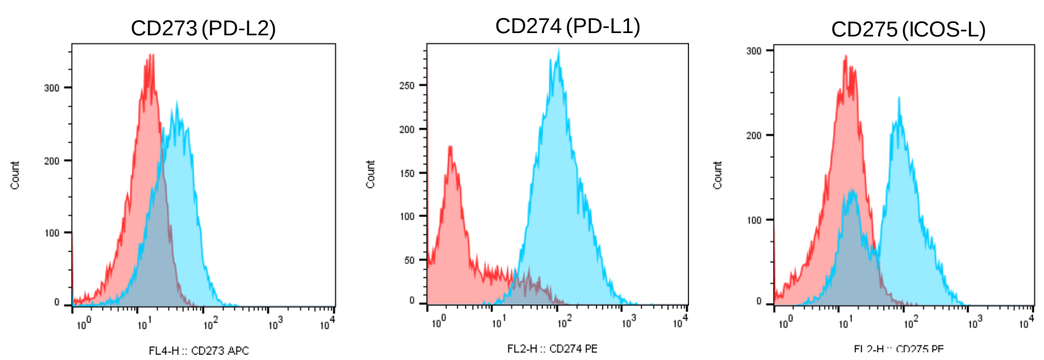

Figure 3. U87 cells express markers of immunosuppression. U87 cells were analyzed by flow cytometry for cell-surface expression of CD273 (PD-L2), CD274 (PD-L1), and CD275 (ICOS-L) using fluorescent antibodies as indicated. The cells exhibited significant levels of immunosuppression marker expression compared to unstained controls.

Results

- Spheroids created by magnetic bioprinting method were highly consistent in density and size, averaging about 525 µm in diameter.
- Clear infiltration of the glioma spheroids by CAR T cells was revealed and quantified over time in the blue channel (405 nm) within ROIs drawn around the perimeter of the spheroids.
- Correlative glioma cell death was measured within the same ROIs in the green fluorescence channel (525 nm).
- U87 cells express markers of immunosuppression indicating that the assay can be utilized to evaluate the effect of checkpoint inhibitors on T cell infiltration and cytotoxicity.

Conclusions

- The use of a fluorescent microscope for image acquisition in the assay provides an object quantification of T cell infiltration and anti-tumor response at microscopic and macroscopic levels in a 3D environment that mimics solid tumors.
- This assay can be scaled up in a 384-well format to provide a high-throughput platform to evaluate combinatorial libraries of therapeutic agents that optimize CAR T cell infiltration and cytotoxicity.
- The degree of T cell infiltration can be further stratified by creating intra-tumoral zones to assess depth of infiltration over time.

References

1) Tseng et al., 2015, “A spheroid toxicity assay using magnetic 3D bioprinting and real-time mobile device-based imaging,” Scientific Reports, 5(1): 13987.