Automated, Image-Based T Cell Mediated Cytotoxicity Assessments using 2D and 3D Target Cell Models

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Introduction

CD3+CD8+ cytotoxic T lymphocytes (CTLs) are the effector cells responsible for T cell mediated cytotoxicity that can kill a variety of cell types, including cancer cells, virus-infected cells, and tumors. Historically, adoptive T cell therapy was limited by the inability to ensure an effective and safe transfer of the effector T cells to the patient. In recent years, the development of treatments avoided use of a patient’s immune system to kill cancer cells, as newer methods were developed using microplate-based optical methods. Due to problems with radioactivity disposal, and low sensitivity due to spontaneous release of the isotope from target cells, newer methods were developed using microplate-based optical methods. These methods rely on the detection of induced cytotoxicity from 2D and 3D plated target cells, as well as visualization of the interplay between CTL and target cells.

The most popular in vitro method to monitor CTL effector on target cells is the cytotoxicity assay, where T cells and target cells are added to a microplate well as co-cultures. Traditionally, toxicity was measured using chromium-51 release from prelabelled target cells. Due to problems with radioactivity disposal, and low sensitivity due to spontaneous release of the isotope from target cells, newer methods were developed using microplate-based optical methods. These methods rely on the detection of induced cytotoxicity from 2D and 3D plated target cells, as well as visualization of the interplay between CTL and target cells.

Here, we demonstrate an automated method to monitor and measure CTL mediated cytotoxicity using digital widefield microscopy. Co-cultured target MDA-MB-231 breast cancer and fibroblast cells were plated in 2D format and 3D bioprinted spheroids, and dosed with a live cell apoptosis/necrosis reagent. T cells, activated using general or directed methods and stained with a red tracking dye, were then added in ratios of 20, 10, 5, or 2.5 to the target cells. The plates were then added to an incubator and shuttered to the digital widefield microscope, using a robot to change the fields every hour where brightfield and fluorescent images were captured. From these images, the cytotoxicity index was calculated for the entire seven days. Visual observation of the kinetic images enabled monitoring of the kinetic images for a total of seven days, as well as visualization of the interplay between CTL and target cells, which can be achieved using digital widefield microscopy.

To determine the kinetics of T cell induced cytotoxicity within the target cells, imaging was performed at regular intervals throughout the incubation period. As extended incubations were required to detect the full cytotoxic effect, T cell addition, brightfield and fluorescent images were captured every four hours for a total of seven days. Figures 3 and 4 demonstrate the iterative cytotoxic effect that T cells, directly activated in the presence of 100% MDA-MB-231 cells and added at a 20:1 ratio, have on 2D and 3D cultured target cells, respectively.

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Image-Based Detection of Co-Cultured Cell Interaction

Over the seven day assay incubation period, activated T cells (red fluorescence) sought out and bound to the target cells in response to T cell binding. When images from the propidium iodide (PI) channel were overlaid with those from the brightfield channel, one can observe that yellow fluorescent signal from the PI necrotic cell probe originates from the same target cell. This overlap provides a quantitative measure of the cytotoxicity effect of T cell-target cell binding.

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Quantification of Target Cell Cytotoxicity

Following image capture, T cell induced target cell cytotoxicity levels were quantified. Object masks were generated by defining the pixels from the PI probe. Object size cutoff values were set to exclude single necrotic T cells, and include small cell aggregates created as the 3D tumour disintegrated or increased in size. The area fraction of the positive pixel was calculated for 2D cultured target cells. For 3D cells, the total PI signal within all object masks per image was quantified as pixels with a value of three or total PI (signal) calculated at each time point were then automatically normalized and plotted to evaluate potential differences in induced target cell cytotoxicity between test conditions.

Conclusions

1. Direct activation of T cells produced a significant increase in cytotoxicity compared to general activation using no target cells, whereas a diminishing effect was evident if the target cells were co-cultured with fibroblasts in the activation process.
2. The target cell concentration was shown to maintain superior cell health over long kinetic runs compared to 2D cell models.
3. Cytotoxicity may be quantified using propidium iodide or brightfield (label-free) methods.