

Validation of an Image-Based 3D Natural Killer Cell Mediated Cytotoxicity Assay

Abstract

An in vitro model for Natural Killer (NK) cell mediated cytotoxicity was developed using a collagen-based scaffold 3D cell culture technology. This technology induced HCT116 cells to aggregate into tumoroids over time which became the target cells during the cytotoxicity assay. Cytotoxicity was assessed by both phosphatidyl serine exposure (apoptosis) and plasma membrane rupture (necrosis) using fully automated workflows. Cytotoxicity was quantified using NK cells alone and with IL-2 stimulation, where a significant increase of cytotoxicity was evident. Cytotoxicity with this model was compared to HCT116 cells adhered to microplates in a conventional 2D format. The 3D model was far superior in both maintaining cell health over time and accurately depicting cytotoxic events.

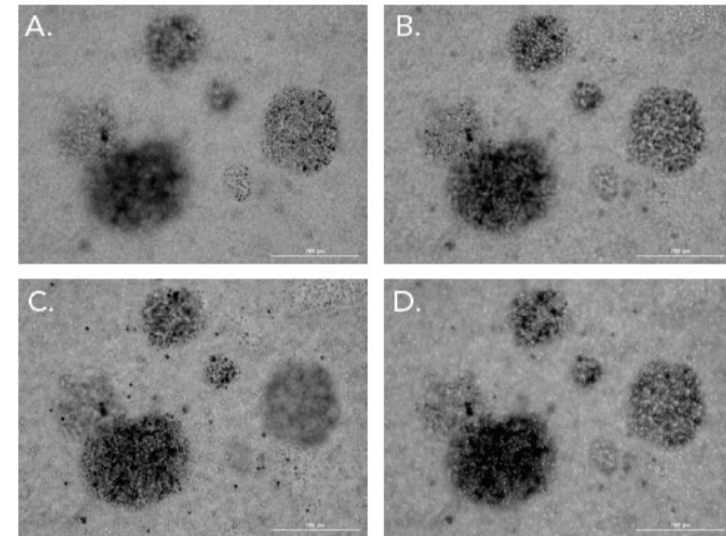


Figure 3. 3D Tumoroid formation conformational imaging. (A.-C.) Brightfield image captured following a 7-day incubation in plate wells using 10x objective at three separate z-heights within the RAFT hydrogel. (D.) Final z-projected image of tumoroids.

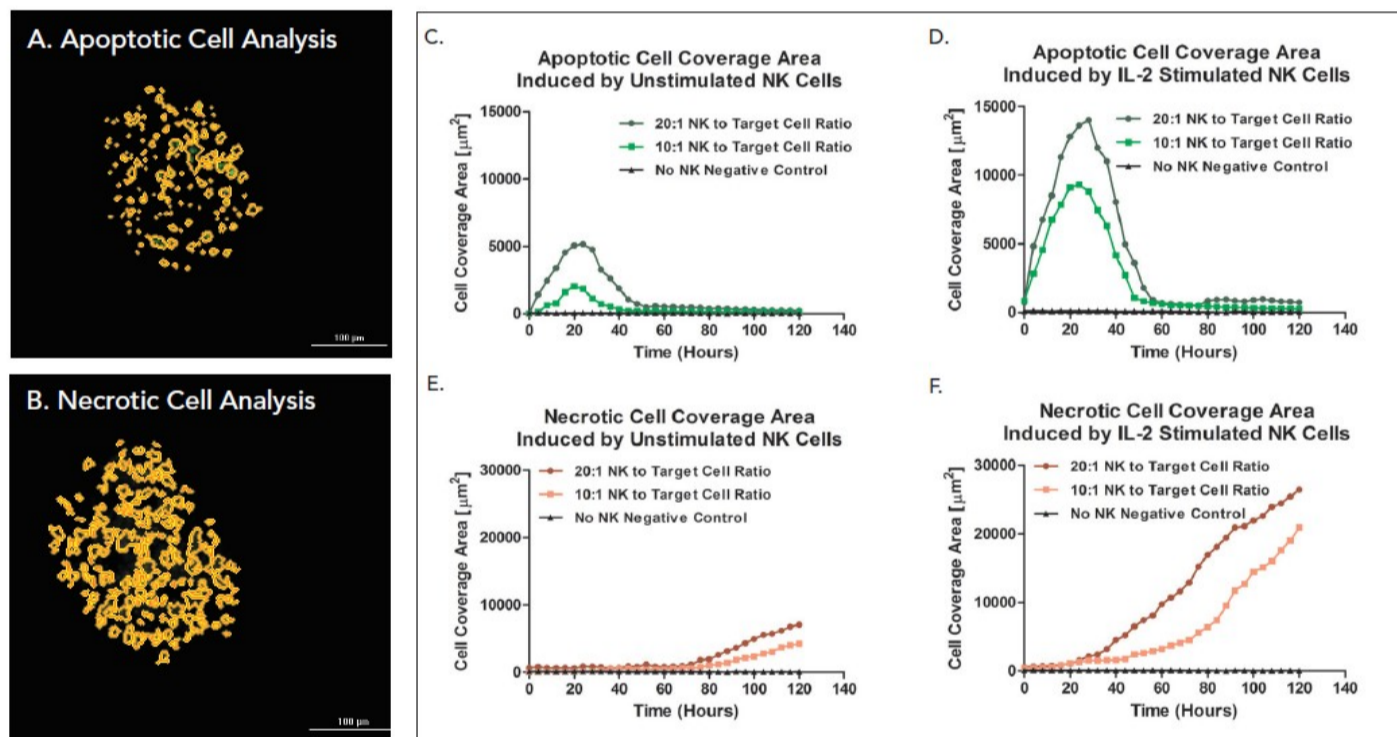


Figure 5. 3D NK CMC analysis. Gen5 placed object masks around (A.) apoptotic or (B.) necrotic cells during individual analysis of zoomed images of a single tumoroid. Total cell coverage area calculated from object masks placed around all apoptotic cells within all tumoroids in the field of view following interaction with (C.) unstimulated or (D.) IL-2 stimulated NK cells; and necrotic cell coverage area following interaction with (E.) unstimulated or (F.) IL-2 stimulated NK cells at each time point.

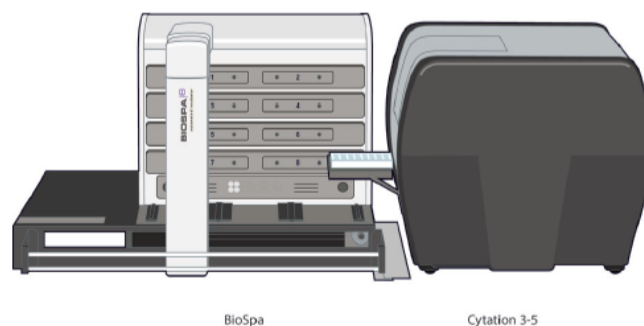


Figure 2. BioSpa™ Live Cell Imaging System consisting of BioSpa 8 Automated Incubator (left) and Cytation™ 5 Cell Imaging Multi-Mode Reader (right) used to automate the cell mediated cytotoxicity assays.

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