APPLICATION NOTE

A1R/AX R Confocal Microscopes
General Analysis Image Analysis Software Module

Confocal Imaging of CAR-T Cell Dynamics Using an Organ-on-a-chip Platform

Evaluation of the immune effect of CAR-T (Chimeric Antigen Receptor T) cell therapy is usually performed using a model organism, which is costly and time consuming. This application note introduces an example of building a simple 3D immune cell-mediated killing assay model using AIM Biotech's 3D cell culture chips, and measuring the immune effects of T cells by *in vitro* imaging. The 3D assay model makes it easy to probe different conditions *in vitro* such as the cancer microenvironment and T cell regulation, and it can be customized in various ways according to the purpose of the research. This assay reproduces the more spatiotemporal dynamics of cells *in vitro* and enables the analysis of immune cell-mediated killing under more physiological conditions as compared to 2D models.

Experiment overview

We evaluated the killing efficiency of antitumor effector T cells (CAR-T cells) by constructing a 3D microenvironment that mimics a tumor using AIM Biotech's idenTx 9 3D cell culture chip and observing and quantitatively analyzing images using a confocal microscope (Fig. 1).
The AIM Biotech idenTx 9 chip used in this experiment consists of 3 fluid channels (Fig. 2A). Firstly, GFP-labeled cancer cells were mixed with Collagen Type I derived from rat tails, and seeded in the central hydrogel channel. The 3 channels can be compartmentalized by having polymerized hydrogel in the center (Fig. 2B, left).
Next, CAR-T cells were added to the medium channel adjacent to the hydrogel channel (Fig. 2B, center). Then, the infiltration of CAR-T cells into the cancer cell-populated hydrogel channel was observed in time series (Fig. 2B, right). In this experiment, 3 types of samples, "Cancer cells only", "Cancer cells + CD133 specific CAR-T cells" and "Cancer cells + Mock transfected T cells," were each prepared and fixed after 24 hours and 120 hours. The infiltration of CAR-T cells was evaluated and the rate of apoptosis of cancer cells was quantified based on the 3D fluorescent images (Fig. 3 and Fig. 4).

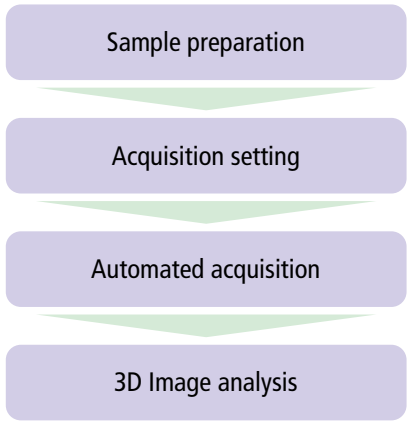
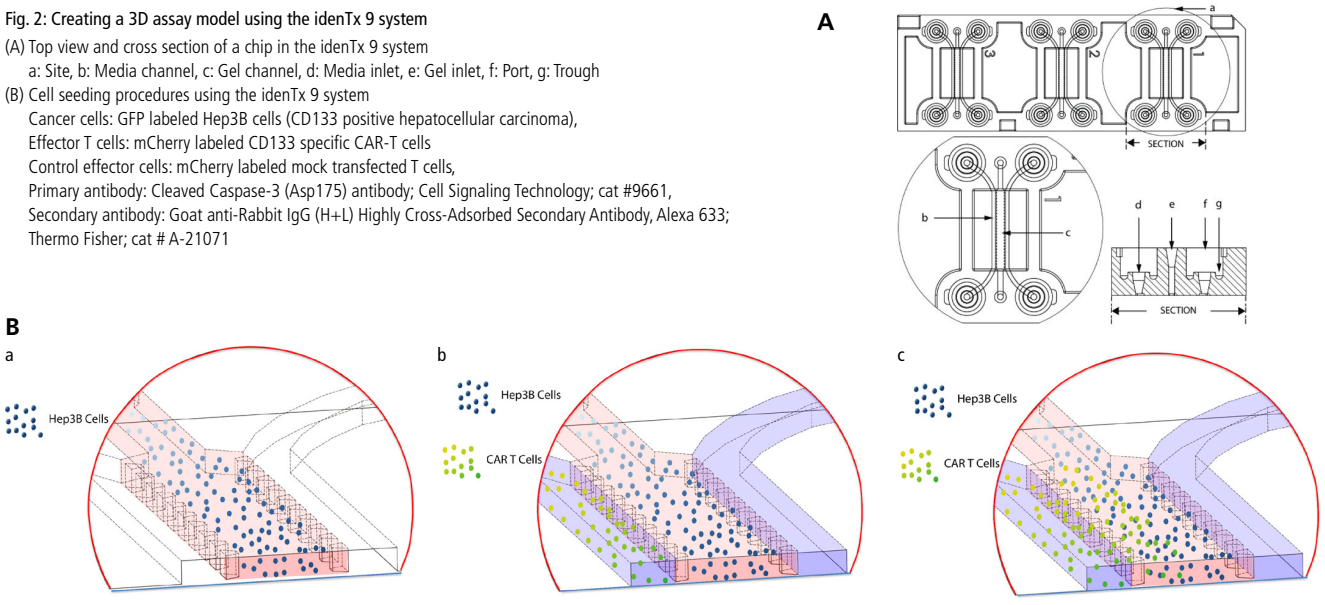


Fig. 1: Experiment workflow

Fig. 2: Creating a 3D assay model using the idenTx 9 system
(A) Top view and cross section of a chip in the idenTx 9 system
a: Site, b: Media channel, c: Gel channel, d: Media inlet, e: Gel inlet, f: Port, g: Trough
(B) Cell seeding procedures using the idenTx 9 system
Cancer cells: GFP labeled Hep3B cells (CD133 positive hepatocellular carcinoma),
Effector T cells: mCherry labeled CD133 specific CAR-T cells
Control effector cells: mCherry labeled mock transfected T cells,
Primary antibody: Cleaved Caspase-3 (Asp175) antibody; Cell Signaling Technology; cat #9661,
Secondary antibody: Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa 633;
Thermo Fisher; cat # A-21071



Chip seeded with Hep3B cells mixed with type I collagen gel.

CAR-T cells loaded into the media channel.

Infiltration of CAR-T cells and their killing ability against target cancer cells were observed.

Infiltration of CAR-T cells induced the apoptosis of cancer cells

CD133 specific CAR-T cells, the effector cells, infiltrated the tumor microenvironment spontaneously within 24 hours and came into contact with the cancer cells (Fig. 3). Also, the dynamics of the fluorescent signals of cancer cells, CAR-T cells, and cleaved Caspase-3 were analyzed, and a correlation was found in the position of their centers of gravity (Fig. 4). In other words, this suggests that CAR-T cells moved towards the cancer cells, stayed near them and induced their apoptosis.

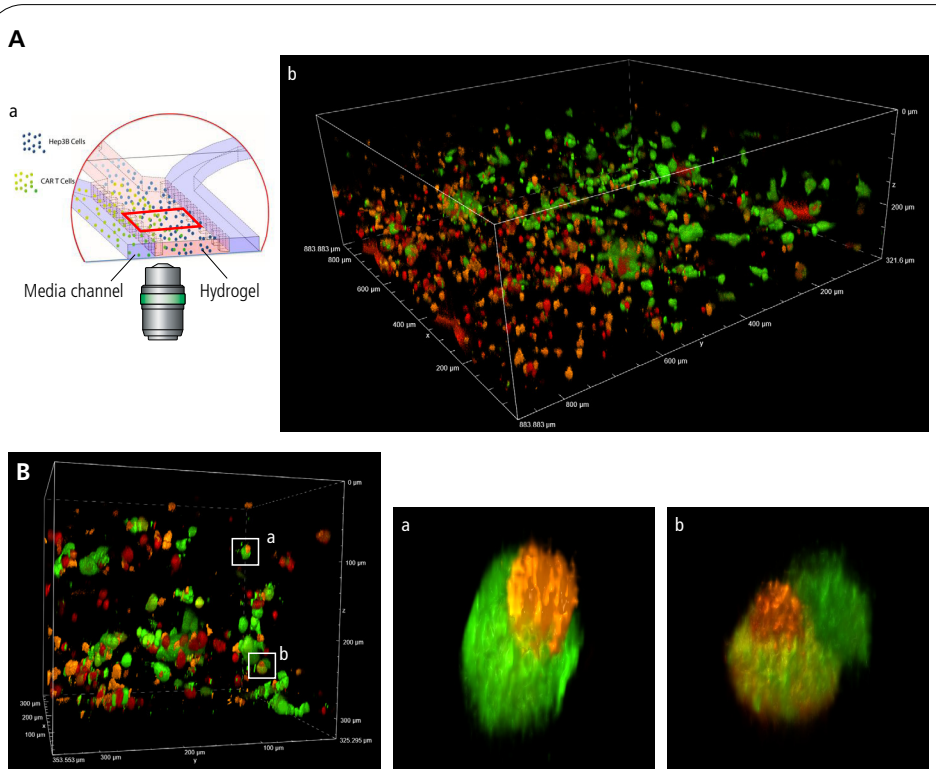


Fig. 3: Dynamics of CAR-T cells and cancer cells after 24 hours

(A) CAR-T cells infiltrating into the hydrogel from the media channel were captured. Image "b" shows a 3D confocal image of the area indicated by the red frame in image "a", captured from the bottom of the chip. By using a water immersion objective with a refractive index close to that of the sample, bright images deep inside the sample could be acquired.
1024 × 1024 pixels, Z-stack (range: 320 µm, step: 1.6 µm)

(B) Cancer cells and CAR-T cells were observed in detail by applying a Nyquist zoom in the central part of the hydrogel channel. Images "a" and "b" show how a CAR-T cell captures a cancer cell.
2048 × 2048 pixels*, Z stack (range: 350 µm, step: 0.8 µm)

Green: GFP labeled Hep3B
Orange: mCherry labeled CD133 specific CAR-T cells
Red: Alexa 633 labeled cleaved Caspase-3
Objective: CFI Apochromat LWD Lambda S 20xC WI
Imaging conditions: resonant scanner

* 2048 × 2048 pixels is supported only by AX R.

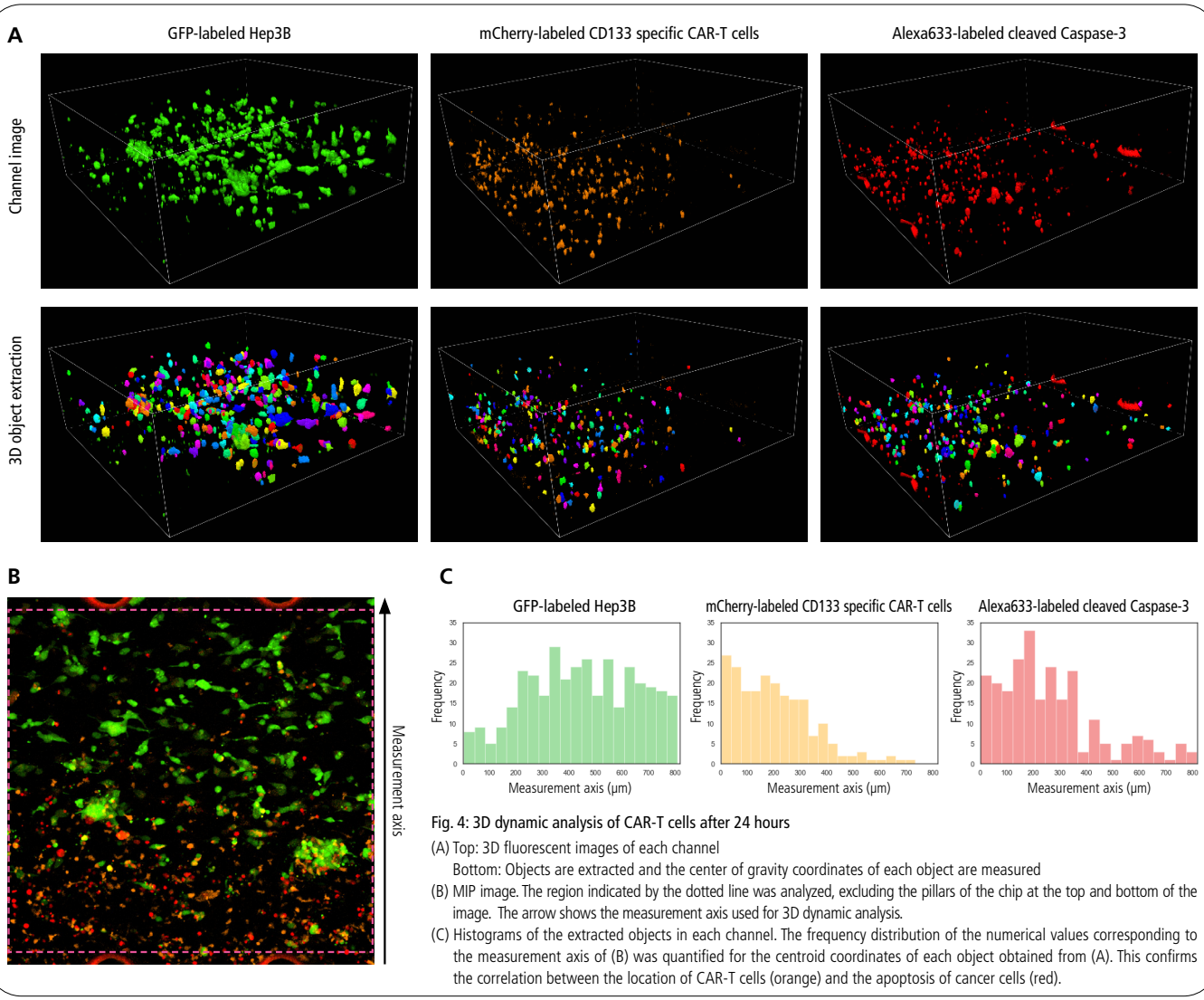


Fig. 4: 3D dynamic analysis of CAR-T cells after 24 hours

(A) Top: 3D fluorescent images of each channel
Bottom: Objects are extracted and the center of gravity coordinates of each object are measured

(B) MIP image. The region indicated by the dotted line was analyzed, excluding the pillars of the chip at the top and bottom of the image. The arrow shows the measurement axis used for 3D dynamic analysis.

(C) Histograms of the extracted objects in each channel. The frequency distribution of the numerical values corresponding to the measurement axis of (B) was quantified for the centroid coordinates of each object obtained from (A). This confirms the correlation between the location of CAR-T cells (orange) and the apoptosis of cancer cells (red).

CD133 specific CAR-T cells have a high killing ability against cancer cells

Next, a comparison of the induction of cancer cell apoptosis by CAR-T cells was performed using 3 types of samples: "Cancer cells only", "Cancer cells + CD133 specific CAR-T cells", and "Cancer cells + Mock transfected T cells". All were fixed after 120 hours. The results revealed that the ratio (volume ratio) of fluorescent signals of cleaved Caspase-3 per cancer cell was significantly higher in the group with CD133 specific CAR-T cells than in the group with mock transfected T cells (Fig. 5). The "Cancer cells only" control group had the lowest apoptosis ratio, as expected.

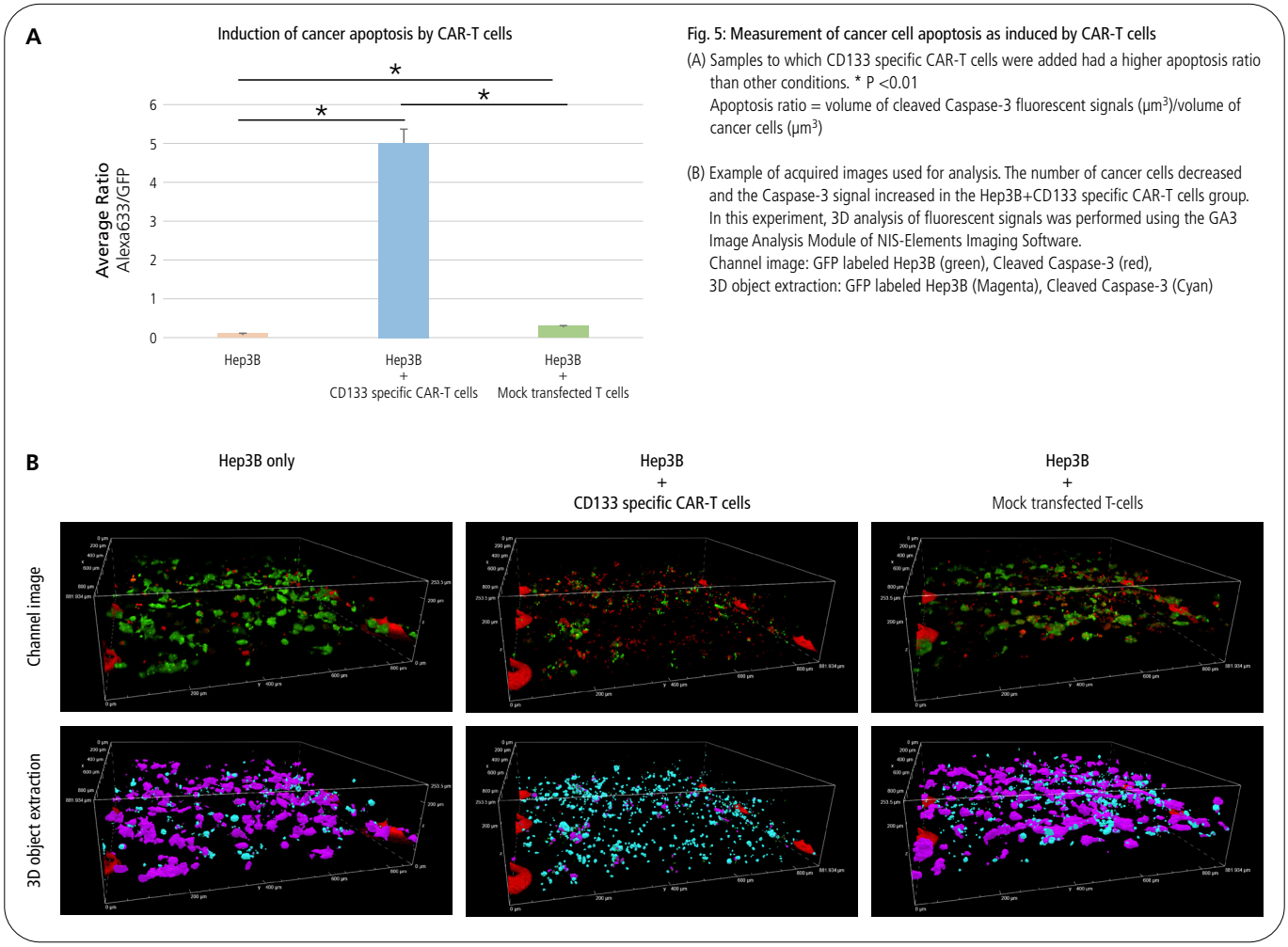


Fig. 5: Measurement of cancer cell apoptosis as induced by CAR-T cells

(A) Samples to which CD133 specific CAR-T cells were added had a higher apoptosis ratio than other conditions. * P <0.01
Apoptosis ratio = volume of cleaved Caspase-3 fluorescent signals (μm^3)/volume of cancer cells (μm^3)

(B) Example of acquired images used for analysis. The number of cancer cells decreased and the Caspase-3 signal increased in the Hep3B+CD133 specific CAR-T cells group. In this experiment, 3D analysis of fluorescent signals was performed using the GA3 Image Analysis Module of NIS-Elements Imaging Software.
Channel image: GFP labeled Hep3B (green), Cleaved Caspase-3 (red),
3D object extraction: GFP labeled Hep3B (Magenta), Cleaved Caspase-3 (Cyan)

Product Information

idenTx 9 Plate (AIM Biotech)

The idenTx 9 fully integrates the capacity of three individual idenTx 3 chips into a standard SBS plate format, enabling 9 simultaneous experiments on a single plate, and rapid scaling up for higher throughput drug screening models. The idenTx 9 retains the same ease-of-use as the idenTx 3 and is a seamless way to progress through feasibility while maintaining the common handling features and laboratory automation compatibility that comes with the standard lab plate format.

AX Confocal Microscope

Achieves high resolution images of 8192 x 8192 pixels, which are four times that of conventional models. With a large diagonal field of view of 25 mm, wide areas of samples can be acquired at once, reducing phototoxicity. An automatic shading correction function enables acquisition of images with uniform brightness.