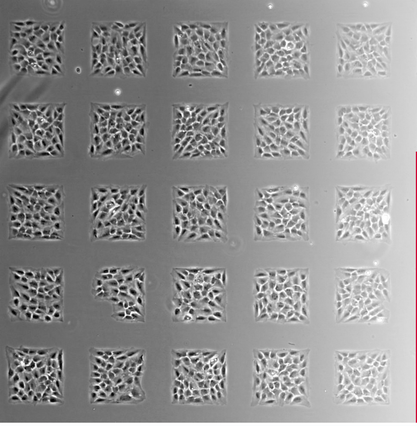

A Micropatterning-Supported High Throughput CAR-T Cell Potency Assay With Single Cell Resolution

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Background

Autologous T cells that express engineered antigen receptors (CAR-T cells) represent a promising new cancer therapy tool. The evaluation of quality, specificity, and killing efficiency (potency) of CAR-T cell populations is crucial for the development of potent and safe patient-specific CAR-T cell therapies.

In contrast to classical T cell potency assays (e.g., Chromium-51), live cell imaging allows to analyze T cell/cancer cell interaction in real time with single cell resolution. However, analysis of confluent cell layers is very time-consuming and therefore not possible in high throughput screens. Additionally, variations in cell size and density require fluorescent labeling for automated T cell and cancer cell registration, which might alter the cell behavior.

To facilitate high throughput label-free analysis of T cell potency in a live cell imaging setup, we generated arrays of homogenously distributed single cancer cells or spheroids. By combining optical analysis and advanced image processing, we were able to evaluate cytotoxic T cell activity over time on a single cell level, without the use of any labeling. Additionally, using matrix-embedded 3D arrays, physiological T cell migration conditions could be mimicked.

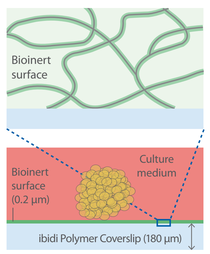
Micropatterning on the Bioinert Surface

The Bioinert Principle

- Thin polyol hydrogel layer, covalently bound to the ibidi Polymer Coverslip #1.5

Features

- Biologically inert—no cell or protein adhesion
- Long-term stable
- Ready-to-use
- Highest optical quality for imaging

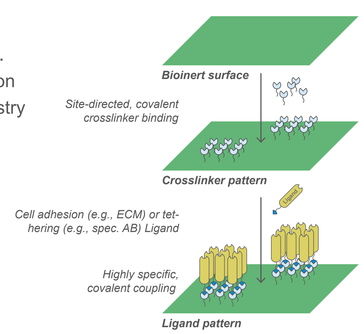


Functionalization

- Specific cell adhesion/tethering for weeks.
- Unspecific cell and molecule immobilization
- Custom-specific adhesion via click chemistry

Optics

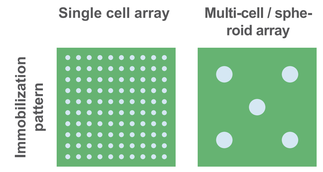
- Very low autofluorescence
- No visibility of µ-Patterns in brightfield
- Optional µ-Pattern fluorescence

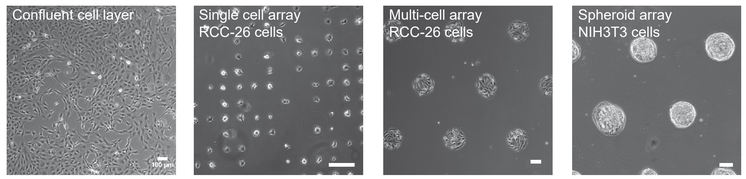


Defined Cancer Cell Arrays on a Micropattern

Using the micropatterning technology, target cells (cancer cells) are immobilized (adhered/tethered) on pads offering either homogenous single cell, or multi cell/cell aggregate distribution.

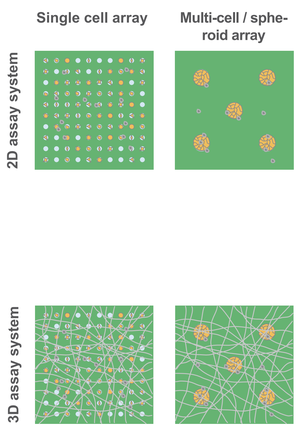
In contrast to a confluent cell layer, this allows for detailed optical analysis on a single cell level.

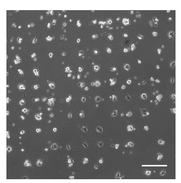




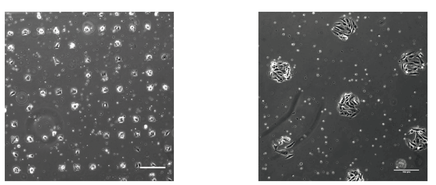
T Cell Potency Assays in 2D and 3D

In order to observe T cell/cancer cell interaction, T cells can either be applied in solution (2D assay), or embedded in a more physiological biological 3D matrix (e.g., collagen).





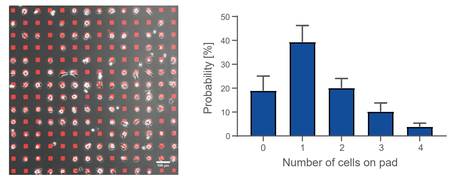
RCC-26 cells immobilized on single cell pads. JB4 effector T cells applied in suspension induce apoptotic body formation of cancer cells.



RCC-26 cells immobilized on single and multi cell pads. JB4 effector T cells applied in collagen matrix induce apoptotic body formation of cancer cells.

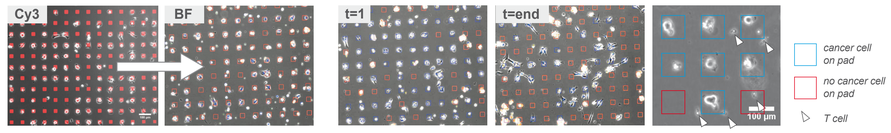
High Throughput T Cell Killing Efficiency Analysis on a Single Cancer Cell Array

Single Cancer Cell Arrays for High Throughput T Cell Killing Efficiency Analysis

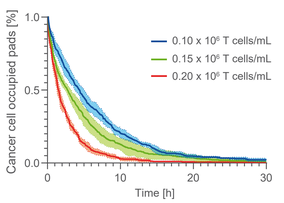


Arrays of single cancer cells were obtained by seeding RCC-26 cancer cells on small adhesive micropads (red). Probabilities were highest for capturing single cells on each adhesion structure (Graph).

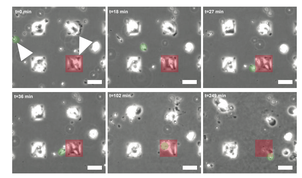
Machine Learning-Based Detection of Cancer Cells on Adhesion Grid Arrays



The array information helps to keep the cell number and positions constant thereby facilitating the analysis. The AI-supported algorithm determines over time whether a cancer cell is attached to a single adhesive pad or not. Like this, the T cell killing efficiency can be calculated.



Characteristic kinetic killing profiles of JB4 T cells applied in suspension and RCC-26 cancer cells distributed on a single cell array.



RCC-26 tumor cell (red) attacked by a JB4 T cell (green).

Conclusion

- We use micropatterning of adhesion ligands to immobilize adherent cancer cells in a single cell or multi-cell manner.
- We observe T cell/cancer cell interaction (2D and physiologically relevant 3D environment) using live cell imaging without the necessity of fluorescent markers.
- We analyze T cell/cancer cell interaction in high throughput using artificial intelligence-based cell recognition (“occupied position” approach).

Outlook

- We aim at applying the micropatterning approach to soluble cancer cells (e.g., B cells via tethering antibodies).
- We aim at analyzing T cell/cancer cell interaction in detail (“T cell tracking”) using higher magnification objectives.
- ACAS (MetaVi Labs and ibidi) Chemotaxis analysis can be used “on top” of cell recognition in order to identify “secondary T cell effects”.