Tunable 3D cell models recapitulating the tumour microenvironment for *in vitro* immuno-oncology assays

Rachel Bleach[1], Lisa Belfiore[1*], Christine Yee[1], Hadi Seyedzadeh[2], Jesse Goyette[2], Katharina Gaus[2] and Marie Besnier[1]

[1] Inventia Life Science, Alexandria, NSW 2015, Australia
[2] School of Biomedical Science, Faculty of Medicine and Health, University of New South Wales, NSW 2031, Australia
* Corresponding author: lisa.belfiore@inventia.life

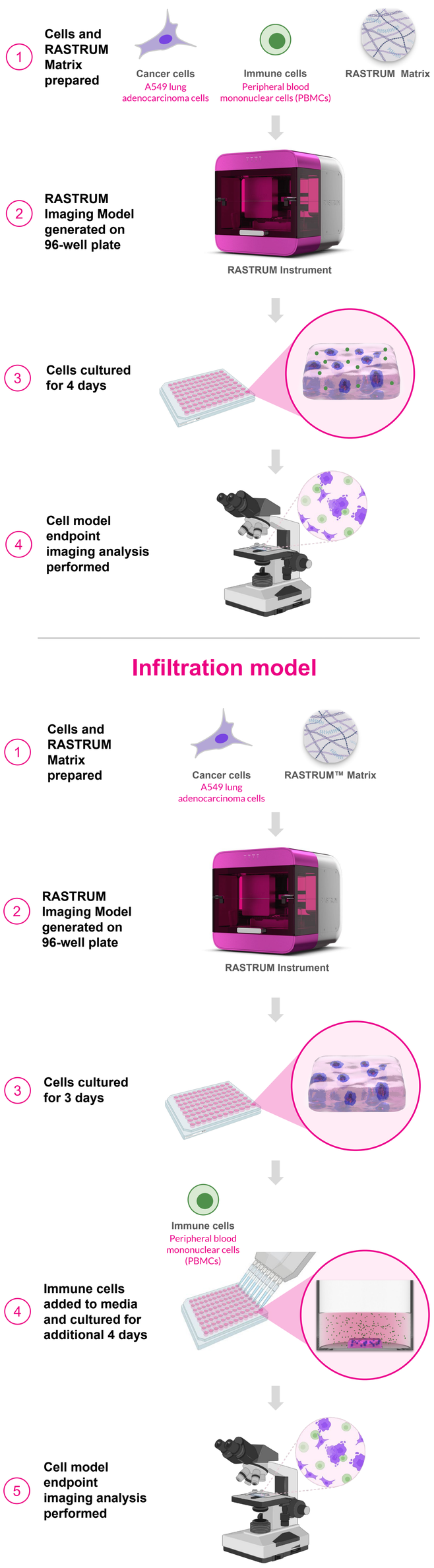
Introduction

Physiologically relevant *in vitro* assays that accurately mimic the tumour microenvironment are crucial for advancing immuno-oncology research. While 2D cell models are routinely used in research due to their simplicity and low cost, the lack of tumour architecture or extracellular matrix (ECM) components in these models limits their biological relevance, which impacts the translation of *in vitro* data through to the clinic. Current 3D cell models based on naturally derived matrices offer a higher level of sophistication, but are typically characterised by limited matrix tunability, non-specific activation of immune cells due to immunogenic matrix constituents, and poor immune cell infiltration into the matrix, limiting the utility of these models in studying complex interactions between immune cells and tumours *in vitro*[1].

Here, we present the RASTRUM™ Platform as a means to create representative 3D cell models for immuno-oncology assays. We describe two different RASTRUM-generated 3D immuno-oncology models: (1) a direct co-culture model, in which tumour cells and immune cells are cultured together within a matrix that recapitulates the tumour microenvironment, and (2) an infiltration model, in which immune cells are added in to tumour cells already within the matrix, recapitulating the *in vivo* process of immune cell recruitment to tumour tissue. The integration of 3D drop-on-demand bioprinting technology with ECM-mimicking matrices allows for the creation of 3D cell models for screening immunomodulatory compounds, assessing immune checkpoint inhibitors, and investigating combination therapies at scale.

Workflow

Direct co-culture model



Infiltration model

Results

Direct co-culture model

Result 1: PBMCs and tumour cells were co-cultured within a matrix that mimics the tumour microenvironment

PBMCs and A549 lung cancer cells were printed at a 4:1 ratio into the RASTRUM Imaging Model using Matrix Px02.28P (~1 kPa), which contained adhesion peptides for fibronectin, laminin and collagen I, and cultured for 4 days in the absence (unactivated) or presence (activated) of CD3/CD28 T cell activator. Immunostaining for pan-cytokeratin (A549s) and CD45 (PBMCs) confirmed the presence of both cell types in the matrix after 4 days, with a greater number of PBMCs present in the activated condition, demonstrating the viability and persistence of PBMCs in the matrix throughout the culture period (**Fig. 1**).

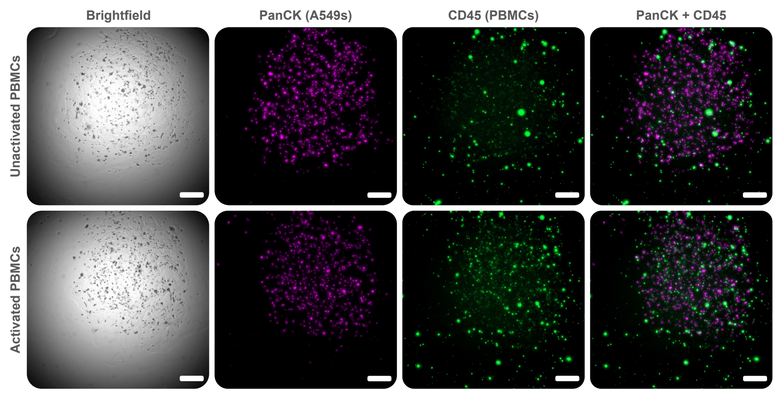


Fig. 1. Brightfield and immunofluorescent images of PBMC/A549 co-culture at day 4. Magenta = pan-cytokeratin (A549s); green = CD45 (PBMCs). Scale bar = 500 µm.

Result 2: Activated PBMCs inhibited the growth of tumour spheroids after 4 days in co-culture

Activated PBMCs associated with tumour cells within the matrix (**Fig. 2A**). Co-culture of activated PBMCs with A549s at a 4:1 ratio, but not at a 2:1 ratio, inhibited tumour spheroid growth compared to unactivated PBMCs, as suggested by a size reduction in A549 spheroids at day 4 post-printing (**Fig. 2B**).

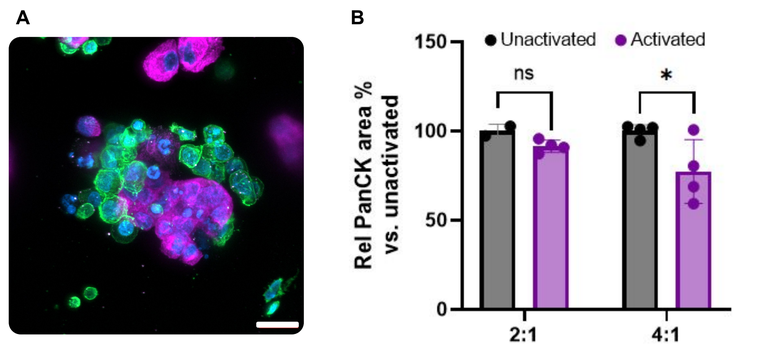


Fig. 2. (A) High-magnification image of activated PBMCs interacting with A549 cells. Magenta = pan-cytokeratin (A549s); green = CD45 (PBMCs). Scale bar = 20 µm. (B) Quantification of growth inhibition as measured by A549 pancytokeratin area after co-culture with unactivated or activated PBMCs at a PBMC:A549 ratio of 2:1 or 4:1.
* $p < 0.05$; ns = not significant.

Result 3: CTL-mediated cytotoxicity and CTL motility were modulated by matrix stiffness

Direct co-culture of cytotoxic T lymphocytes (CTLs) and OVA+ CHO cells in RASTRUM Matrix[2] revealed greater CTL cytotoxicity against OVA+ CHO cells in a softer matrix at an effector:target ratio of 5:1 (**Fig. 3A**). The average CTL speed, a measure of immune cell motility within the matrix, was observed to be higher in the soft matrix relative to the stiff matrix (**Fig. 3B**).

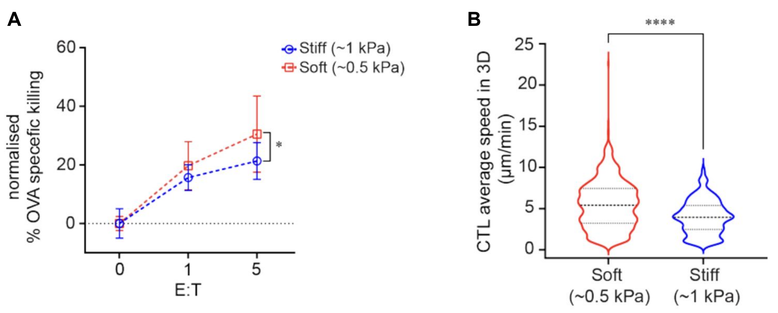


Fig. 3. (A) CTL cytotoxicity against OVA+ CHO cells in stiff and soft matrices at different effector:target (E:T) ratios. (B) Average CTL speed in soft and stiff matrices as measured using a Zeiss CD7-900 microscope. * $p < 0.05$; **** $p < 0.0001$. Data adapted from Seyedzadeh, H. (2023).

Infiltration model

Result 4: CTLs infiltrated the matrix and associated with tumour cells over 4 days in culture

A549 cells were cultured in RASTRUM Matrix Px02.28P for 3 days before 50,000 activated CTLs were added to the media. At day 4 post-CTL addition, cell staining and z-stack imaging revealed CTL infiltration into the matrix and association of CTLs with A549 cells (**Fig. 4**).

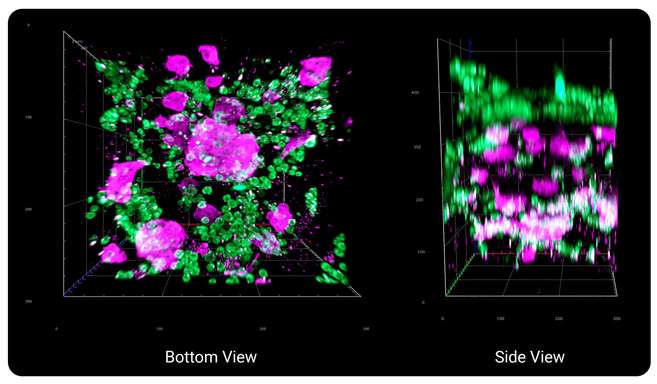


Fig. 4. Representative z-stack of infiltration model 4 days post-addition of CTLs (50,000 per well). Presented views are from the bottom looking up through the matrix (left) and side view of the matrix (right). Magenta = pan-cytokeratin (A549s); green = CD45 (PBMCs). Numbers on axes indicate dimensions in µm.

Result 5: CTLs inhibited tumour cell growth in a concentration-dependent manner

Infiltrating CTLs exhibited a growth inhibition effect against A549 spheroids compared to untreated spheroids, and inhibition occurred in a CTL density-dependent manner (**Fig. 5**).

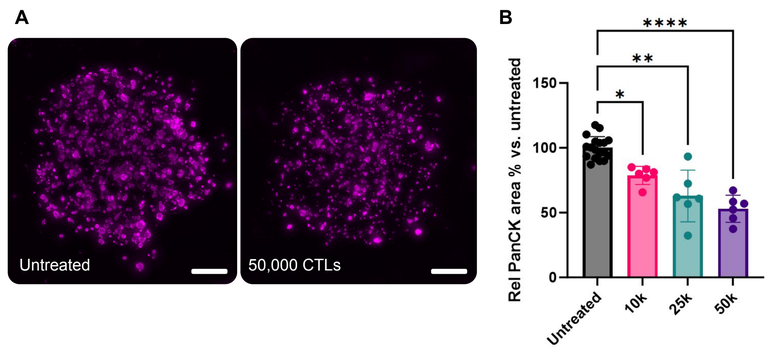


Fig. 5. (A) Representative images of A549 spheroids stained for pan cytokeratin after 3 days of culture with or without addition of cytotoxic T cells (CD8+ cells) (green channel for CD45 removed for analysis). Scale bar = 500 µm. (B) Quantification of pan CK staining (for A549 cells) after treatment with 10,000, 25,000 or 50,000 CTLs. * $p < 0.05$.

Summary

Here, we describe two different 3D cell culture models to study immune cell association with tumour cells, immune cell-mediated anti-tumour effects, and immune cell infiltration into the matrix. The generation of these immuno-oncology models using the RASTRUM Platform enables:

- Compatibility with both tumour cell and immune cell culture to allow for the creation of co-cultures that recapitulate the tumour microenvironment
- Tunability of the matrix to modulate composition and stiffness to influence immune cell cytotoxicity and motility
- Infiltration of immune cells into the matrix to facilitate immune–tumour cell associations and study anti-tumour effects

References

1. Mu, P. et al. (2023) Newly developed 3D in vitro models to study tumor–immune interaction. J Exp Clin Cancer Res 42, 81.
2. Seyedzadeh, H. (2023) Investigating how cell extrinsic and intrinsic factors affect cytotoxic T lymphocyte function. (Doctoral Dissertation, UNSW). DOI: https://doi.org/10.26190/unsworks/24732