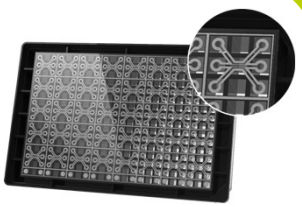
A microfluidic 3D endothelium-on-a-chip model to study transendothelial migration of T cells in health and disease

L. de Haan[1], J. Suijker[1], R. van Roey[1], N. Berges[2], E. Petrova[2], K. Queiroz[1], W. Strijker[1], T. Olivier[1], O. Poeschke[2], S. Garg[2] & L.J. van den Broek[1]

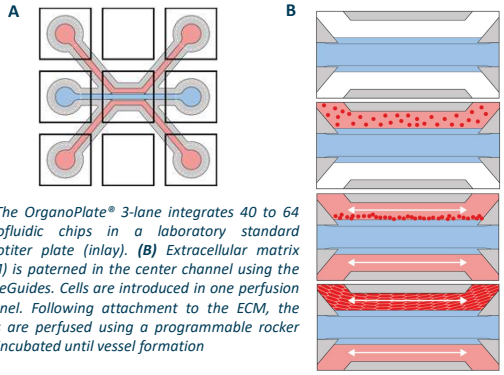
1. Mimetas BV, de Limes 7, 2342 DH Oegstgeest, The Netherlands.
2. Merck Healthcare KGaA, Frankfurter Str. 250, 64293 Darmstadt, Germany.

The OrganoPlate®



T-cells recruitment is a crucial aspect of inflammation. It involves the transport and stable arrest to vessel walls at the site of inflammation, followed by extravasation and infiltration into tissue. We describe an assay to study 3D T cell dynamics under flow in real time using a high-throughput, organ on a chip platform that allows unimpeded extravasation of T cells. We envision routine use of this assay in the study of immunological disorders, immuno-oncology and the development of novel immunotherapeutics.

Modelling Endothelium-on-a-chip in the OrganoPlate® 3-lane



(A) The OrganoPlate® 3-lane integrates 40 to 64 microfluidic chips in a laboratory standard microtiter plate (inlay). (B) Extracellular matrix (ECM) is paterned in the center channel using the PhaseGuides. Cells are introduced in one perfusion channel. Following attachment to the ECM, the chips are perfused using a programmable rocker and incubated until vessel formation

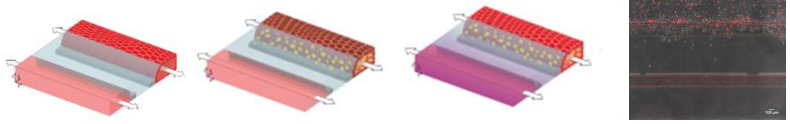
Co-culture of endothelial cells and T cells

Step 1: Construct an 3D endothelial vessel

Step 2a: Introduce compound and/or immune component

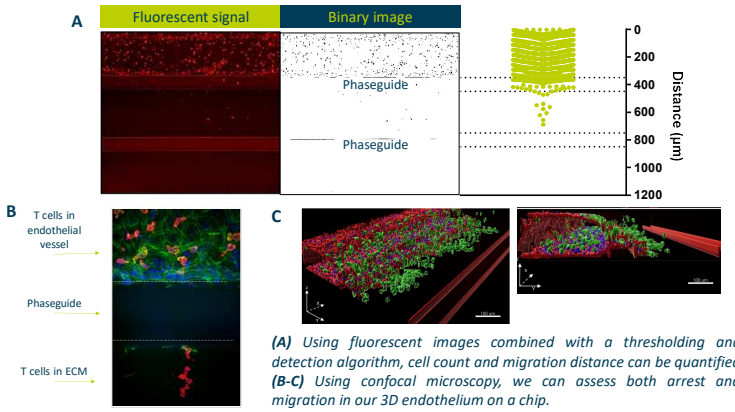
Step 2b: Introduce a trigger (chemokine, purple hue)

Step 3: Live track labelled T cells



Once the vessel is formed (step 1), it can be treated with compounds (step2a), for example to induce vascular inflammation. The vessel is then perfused with labelled T cells (step 2a). A trigger can be added to the bottom channel (step 2b). Migration of labelled T cells can be followed in real time using fluorescent microscopy (step 3)

Quantification of T cells migration

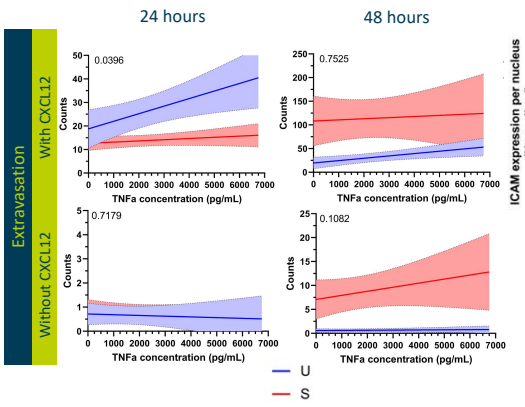


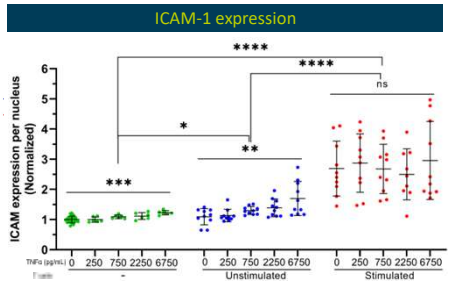
(A) Using fluorescent images combined with a thresholding and detection algorithm, cell count and migration distance can be quantified (B-C) Using confocal microscopy, we can assess both arrest and migration in our 3D endothelium on a chip.

TNFα-mediated vascular inflammation

Treatment of the endothelial vessel with TNFα resulted in a dose dependent increase of ICAM-1 expression for mono-cultured vessels and vessels co-cultured with unstimulated T cells. Co-culture with (CD3/CD28)-stimulated T cells resulted in increased ICAM-1 expression independent of TNFα exposure.

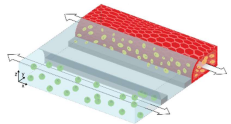
In the presence of CXCL12, migration of unstimulated T cells was increased by TNFα in a dose-dependent manner. Migration of stimulated T cells was increased irrespective of TNFα concentration, with most migration happening after 48 hours of co-culture.

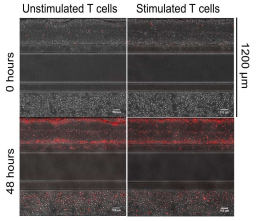




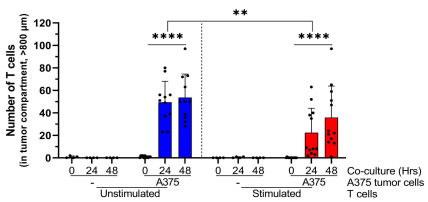
The number of migrating T cells in response to TNFα concentration was analyzed using regression analysis, showing different responses for unstimulated (blue) and stimulated (red) T cells depending on duration of co-culture and the presence of CXCL12. Shown are fitted lines, the computed 95% confidence bands as well as p-values indicating similarity of regression curves.

T cell infiltration towards tumor compartment





Addition of ECM-embedded melanoma cells to the the basolateral channe resulted in the attraction of T cells to towards the tumor compartment. Quantification of T cells migration shows a difference between unstimulated and stimulated T cell populations.



Conclusions

We developed an assay for the routine study of trans-endothelial T cell migration in 3D using the OrganoPlate®.

A perfused vascular component allows stable arrest of T cells, followed by extravasation and migration into a 3D extracellular matrix.

These events can be modulated by inflammation and the presence of chemotactic gradients. Co-culture with tumor cells enables physiologically relevant study of T cell infiltration into tumor microenvironments.

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