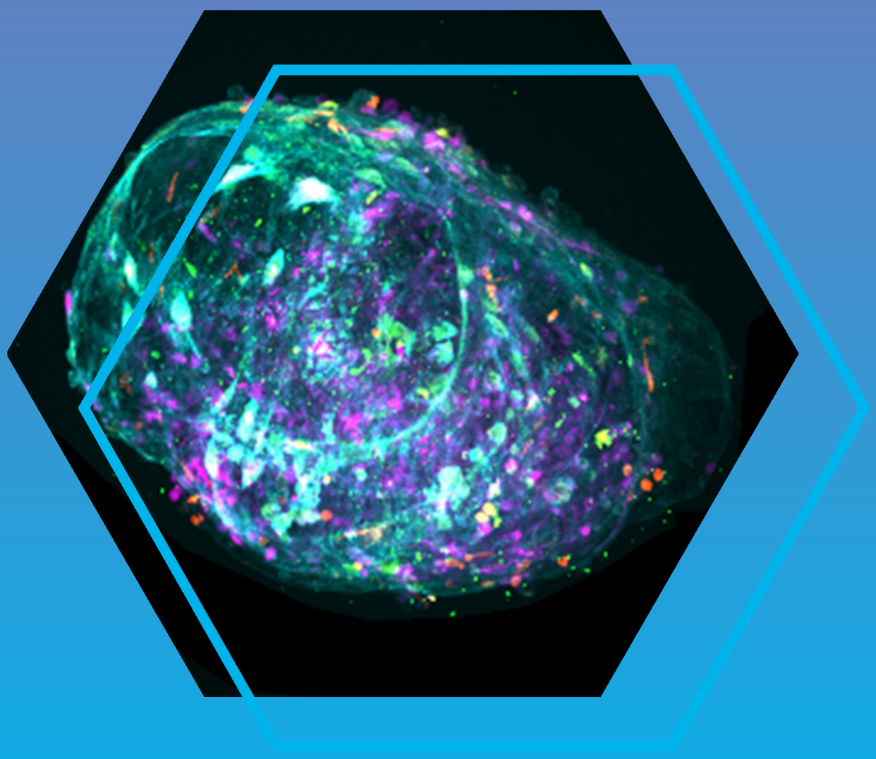
Application Note

Cancer-on-chip for drug testing and immune response

Modelling of the PDAC tumor microenvironment in a vascularized spheroid-on-chip model



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Abstract

Pancreatic ductal adenocarcinoma (PDAC) remains one of the most difficult cancers to manage, marked by a poor prognosis and resistance to standard treatments. A hallmark of PDAC is its highly complex tumor microenvironment (TME), which is abundant in immune cells and cancer-associated fibroblasts. Dynamic42's spheroid-on-chip technology introduces an innovative in vitro platform to replicate the intricate features of PDAC. This application note outlines the creation of a perfused three-dimensional (3D) PDAC model within a microfluidic chip system. The model effectively captures the dynamic interactions between PDAC cells and their TME, establishing an optimal framework for assessing drug efficacy and immune response. By incorporating perfusion, it mimics physiological conditions, enabling real-time investigations into drug delivery and cellular behavior.

Highlights

- The three-channel chip enables culturing of tumor spheroids under a vascular barrier.
- Incorporation of primary fibroblasts forms a fibrotic shell around tumors.
- Drugs can be administered through the vasculature.
- Drugs can be assessed for specific toxicity against spheroids.
- Primary monocytes applied through the vasculature migrate into PDAC spheroids.
- Invading monocytes differentiate into M2-like macrophages.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the most common and lethal form of pancreatic cancer, accounting for over 90% of pancreatic malignancies. It is characterized by rapid progression, high metastatic potential, and resistance to conventional therapies. One of the greatest challenges in addressing PDAC lies in its dense stromal environment, which creates physical and biochemical barriers that impede drug delivery and immune system response. With an exceptionally low five-year survival rate—hovering around 10%—there is an urgent need for advanced, patient-specific models to better understand this devastating disease and accelerate the development of effective treatments.

Cancer spheroids are three-dimensional clusters of cancer cells that closely replicate the architecture and behavior of tumors, offering a more accurate model than traditional two-dimensional cell cultures. They mimic key features of the tumor microenvironment, including cell-cell and cell-matrix interactions, hypoxia, and nutrient gradients, making them invaluable tools for cancer research. Spheroids are widely used to study tumor growth, metastasis, and therapy response under conditions that resemble those in the human body. Moreover, they provide a platform for testing drug efficacy and evaluating combination therapies in a controlled and physiologically relevant setting.

Organ-on-chip technology introduces a revolutionary approach to modeling human cancers in vitro. By replicating critical aspects of the tumor microenvironment, such as the extracellular matrix, vascular networks, and cellular heterogeneity, organ-on-chip devices enable researchers to study complex tumors in a controlled yet physiologically relevant context. These platforms facilitate investigations into the interactions between cancer cells and the stromal compartment, mechanisms of drug resistance, and the testing of novel therapeutic strategies.

Additionally, organ-on-chip models allow the incorporation of patient-derived cells, making it possible to study individual tumor characteristics and develop personalized treatment regimens. Importantly, such cancer-on-chip models must incorporate sufficient complexity to faithfully replicate PDAC-like tumors.

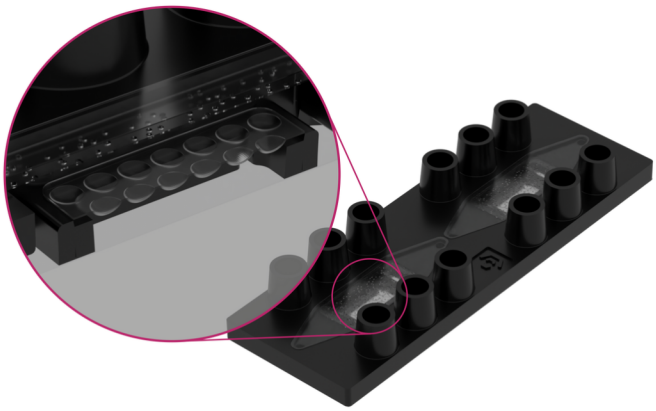
Dynamic42 has developed an advanced chip that combines the complexity of spheroids with organ-on-chip models, allowing for unprecedented advancements in cancer model development. This application note highlights the use of PDAC spheroids beneath a vascular barrier, enabling dynamic drug treatment and the assessment of immune-cancer interactions.

Materials and Methods

The materials and methods outlined in this application note closely follow those outlined in Deipenbrock et al., 2025. Any modifications or adaptations made to the protocol are clearly specified.

Hardware & Consumables

/ Peristaltic pump
/ Biochip BC003_8PET_MC
/ 2-Stop Tubing
/ Connectors (Adapter)
/ Plugs
/ Reservoirs (1 ml)
/ Ultra low adhesion 96 well plates



BC003 three-channel biochip

Cells

/ HUVEC human ubillical cord vascular endothelial cells
/ PANC-1 Tumor cell line
/ PSC Pancreatic stellate cells - Primary PDAC associated Fibroblasts
/ Primary monocytes

Media and reagents for cell and on-chip culture

/ EGM2 endothelial growth medium
/ DMEM F12
/ DMEM
/ Qualified FBS
/ Rat Collagen
/ Matrigel®
/ Penicillin–streptomycin

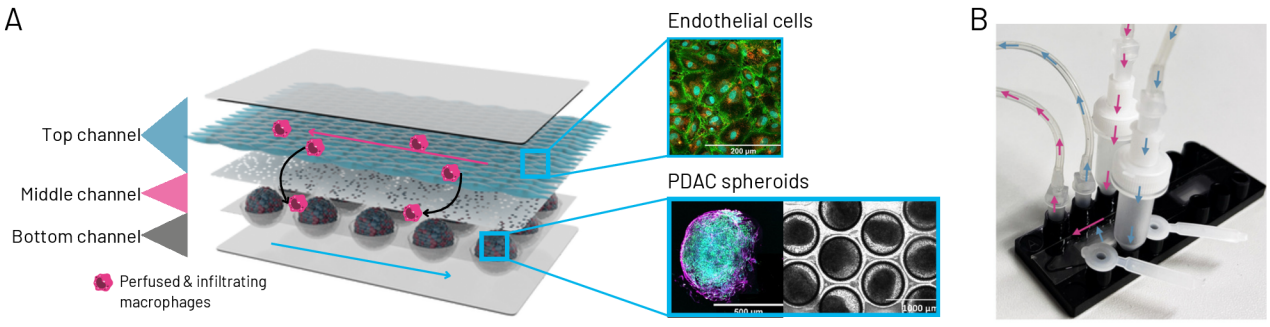
Results

1. Development of PDAC-on-Chip Model Using the Dynamic42 3-Channel Biochip BC003

The PDAC-on-chip model was established using the Dynamic42 three-channel biochip (BC003), which features a polyethylene terephthalate (PET) membrane with 8 µm pores (median density: 1 × 10/cm^2) separating the upper and middle channels, and a microcavity (PC) membrane with 2 µm pores for the middle and lower channels (Fig 1A).

To prepare the vascular compartment, human umbilical vein endothelial cells (HUVECs) were seeded into the upper channel and cultured statically for 3 days until confluency was achieved (Fig 1A). Simultaneously, pancreatic cancer cells (PANC-1) and tumor derived pancreatic stellate cells (PSC) were co-seeded into ultra-low adhesion U-bottom plates. Over 4 days, the two cell types self-assembled into PDAC spheroids.

On Day 4, the spheroids were mixed with 2.5% Matrigel (v/v) and introduced into the microcavities of the middle channel (Fig 1A). Circular perfusion was initiated using a peristaltic pump (25 µl/min), with the upper channel simulating blood flow and the lower channel providing sustained nutrient delivery (Fig 1A, 1B). At 72 hrs of perfusion, the endothelial layer (Fig 1C) and PDAC spheroids, as characterized by a proliferative PANC-1 core encased in a fibrotic shell (Fig 1D), are still intact. Drug treatments or immune cells were administered through the vascular channel for therapeutic evaluations and immune polarization assays.



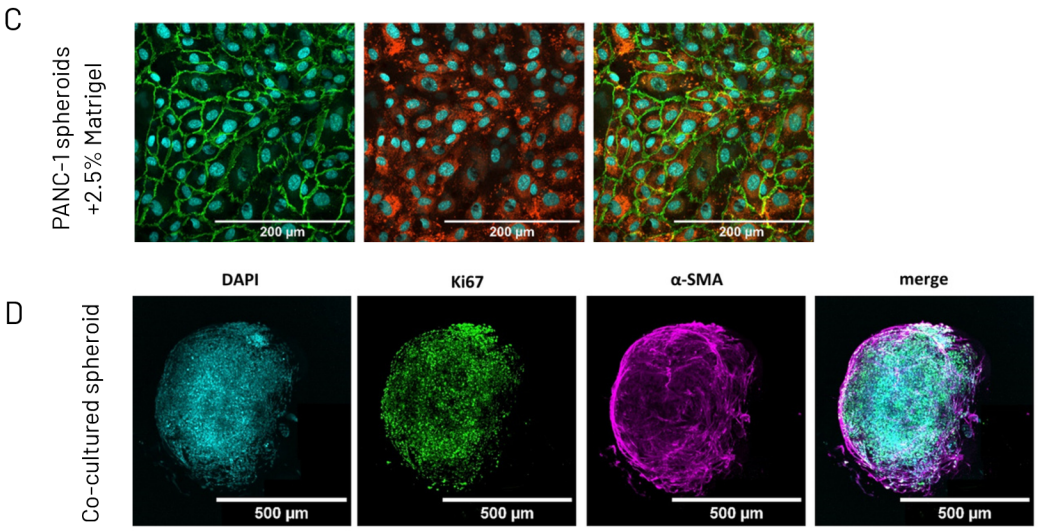


Figure 1: (A) Schematic illustration of the PDAC model. Arrows depict the flow direction in the top (magenta) and the bottom (blue) channel. (B) Perfusion setup for the PDAC model. The tubing connects ports of the top and the bottom channel to create two independent perfusion circles. The middle channel is sealed. Arrows indicate the media flow direction in the top (magenta) and the bottom (blue) channel during perfusion. (C) The vasculature remains stable under flow with an addition of 2.5% matrigel in the presence of PANC-1 spheroids. Stained the endothelial markers VE-cadherin (green), von Willebrand factor (vWF) (red) and the nuclear dye DAPI (blue) were stained. Shown as MIP of a Z-stack. Scale bar is 200 µm (D) IF staining of 3D PDAC spheroids after 72 h cultivation on the biochip shown as maximum intensity projection (MIP) of a Z-stack. DAPI-staining of nuclei (blue); staining of the proliferation marker Ki67 (green); staining of fibroblast marker α-SMA (purple); merging of all three channels. Scale bar is 500 µm.

2. Evaluating Vorinostat (SAHA) in a PDAC-on-Chip Model

Vorinostat (SAHA), a histone deacetylase (HDAC) inhibitor approved for the treatment of cutaneous T-cell lymphoma (CTCL), demonstrates selective cytotoxicity against cells overexpressing class I HDACs and HDAC6, while sparing healthy cells. This selective effect has been observed across multiple malignancies, including pancreatic ductal adenocarcinoma (PDAC).

To evaluate the efficacy of SAHA in a PDAC-on-chip model, increasing concentrations of the compound were applied. Importantly, Vorinostat was not adsorbed by the biochip (Fig 2B). At 72 h treatment did not compromise endothelial barrier integrity (Fig 2A, C), as confirmed by assessing vascular barrier function and morphology. However, SAHA exhibited a dose-dependent cytotoxic effect on the underlying PDAC spheroids (Fig 2D). This suggests that SAHA retains its antitumor potency in a dynamic, physiologically relevant 3D microenvironment, while preserving vascular compartment stability.

The findings highlight the utility of organ-on-chip technology for preclinical drug testing, offering an advanced platform for evaluating the therapeutic potential of HDAC inhibitors like SAHA in PDAC. This model enables comprehensive assessment of drug responses in a controlled, human-relevant setting, paving the way for more accurate predictions of clinical outcomes.

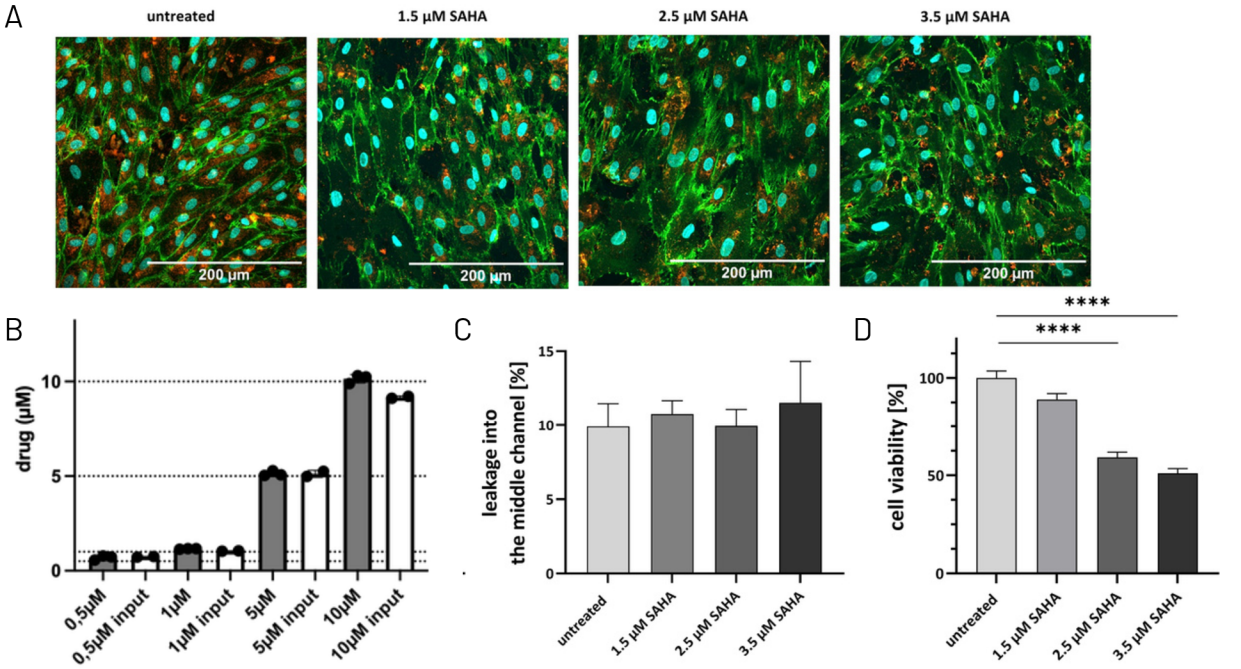


Figure 2: Dynamic administration of the HDAC inhibitor SAHA in the PDAC biochip model. (A) Investigation of the influence of increasing SAHA concentrations on the vasculature integrity. VE-cadherin (green), von Willebrand factor (red) as marker of the endothelial cells, DAPI for staining the cell nuclei (blue). Shown as MIP of a Z-stack. Scale 200 µm. (B) SAHA was introduced at indicated concentrations into the perfusion of a cell free BC003 Biochip. At 24 h SAHA concentration was determined by LCMS. Grey bars indicate concentrations after perfusion. White bars indicate concentration of the input controls. Experiment was run in triplicate, controls in duplicate. © (C) Permeability assay of HUVEC layer after treatment with SAHA for 72 h. FITC dextran assay was performed to measure the barrier integrity of the HUVEC layer after 72 h treatment with 1.5, 2.5, or 3.5 µm SAHA. FITC dextran solution was added into the top channel. After 1 h the leakage into the mid-channel was determined via fluorescence measurement. (D) Investigation of the viability of the 3D PDAC spheroids in the biochip in the presence of SAHA after 72 h administration. Error bars indicate the standard error of the mean, n=3;**** = $p \leq 0.0001$.

3. Immune Invasion and Polarization in a PDAC-on-Chip Model

M2-polarized macrophages play a critical role in the tumor microenvironment of pancreatic ductal adenocarcinoma (PDAC). To investigate immune polarization in a PDAC-on-chip model, peripheral blood mononuclear cell (PBMC)-derived monocytes were perfused through the vascular channel. Monocyte migration across the endothelial barrier into the tumor spheroids was assessed to evaluate immune cell recruitment.

The monocytes successfully traversed the endothelial barrier and infiltrated the fibrotic shell surrounding into the tumor spheroids (Fig 3A). Within this microenvironment, the monocytes exhibited an increase in the expression of M2-associated markers, CD163 and CD206. Concurrently, the expression of M1 markers, such as HLA-DR and CD86, was significantly reduced, indicating a polarization toward an M2 macrophage phenotype (Fig 3B).

These results demonstrate the capability of the PDAC-on-chip model to recapitulate immune cell recruitment and polarization within a dynamic and physiologically relevant tumor microenvironment. This system provides a valuable platform for studying immune modulation in PDAC and testing therapeutic strategies targeting macrophage polarization.

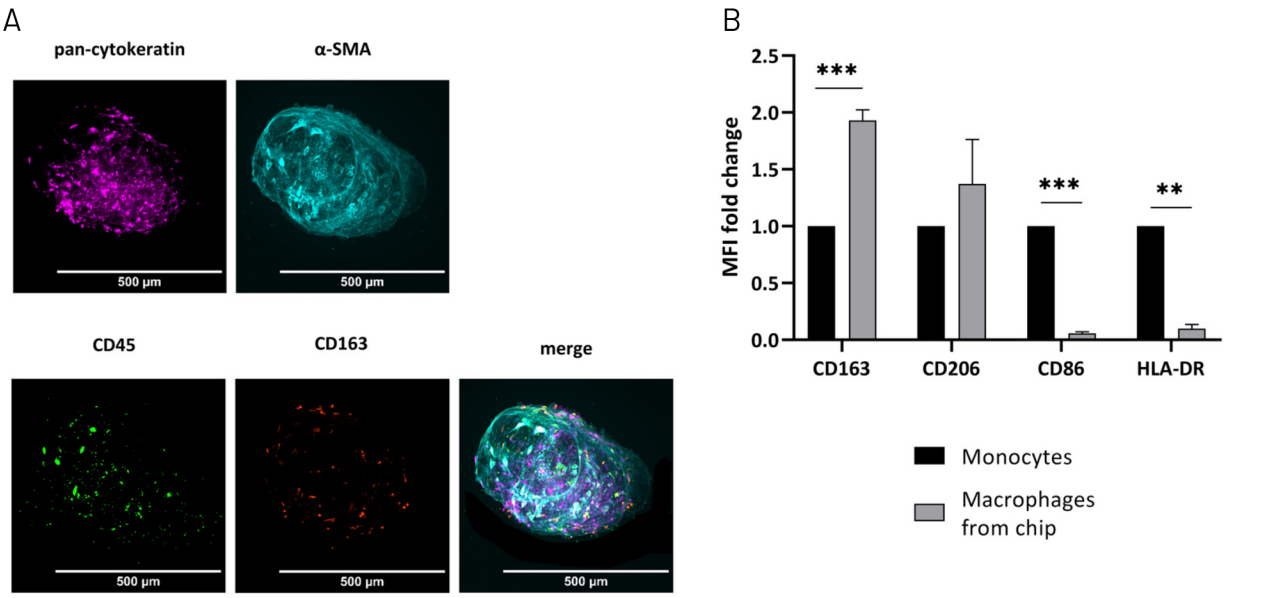


Figure 3: Polarization of monocytes after perfusion in the biochip. (A) IF staining of co-culture spheroids after perfusion with primary monocytes over the endothelial layer. Co-culture spheroids were seeded into the biochip and primary monocytes were perfused over the endothelial layer in the TOP channel. After 72 h of perfusion the co-culture spheroids were stained for the tumor cell marker pan-cytokeratin (purple), the fibroblast marker α-SMA (blue), the immune cell marker CD45 (green), and the M2 marker CD163 (red); merging of all four channels. Shown as MIP of a Z-stack. Scale bar is 500 µm. (B) Marker characterization of polarized macrophages. Polarization after infiltration into PDAC spheroids was analyzed by flow cytometry 72 h after perfusion with monocytes in the biochip. Macrophages identified as CD11b + and CD45 + were analyzed for surface expression of CD163, CD206, CD86, and HLA-DR. Infiltrated cells were compared to unstimulated monocytes as controls. Error bars indicate the standard errors of the mean of three independent experiments, with **= $p \leq 0.01$; *** = $p \leq 0.005$.

Conclusion

PDAC remains one of the most difficult cancers to treat due to its highly complex TME, characterized by abundant immune cells and cancer-associated fibroblasts.

The Dynamic42 Organ-on-Chip technology offers a groundbreaking solution, enabling the development of a 3D, perfused PDAC-on-chip model within a microfluidic platform. This innovative model accurately mimics the dynamic interactions between PDAC cells and their TME, incorporating vascular flow and nutrient delivery to replicate physiological conditions. It facilitates real-time investigation of drug efficacy, immune cell interactions, and therapeutic strategies.

The Dynamic42 PDAC-on-chip model is a transformative tool for pancreatic cancer research, offering a physiologically relevant, human-centered platform. It allows for comprehensive drug evaluation and immune interaction studies, addressing limitations of traditional models and paving the way for improved therapeutic strategies. This innovative system holds promise for advancing preclinical research and developing personalized treatments for PDAC.

Key takeaways

- Drug Testing: Vorinostat (SAHA), a histone deacetylase (HDAC) inhibitor, exhibited dose-dependent cytotoxicity against PDAC spheroids while preserving endothelial barrier integrity, confirming its efficacy in a 3D microenvironment.
- Immune Modulation: Monocytes successfully infiltrated the tumor spheroid fibrotic shell and polarized into M2 macrophages, reflecting the immunosuppressive phenotype characteristic of PDAC's TME.

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