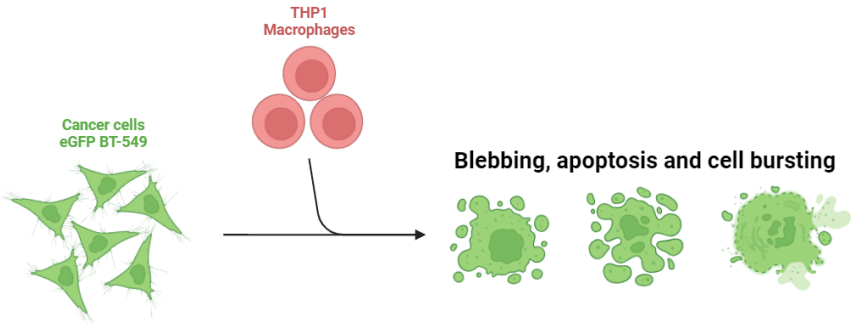
Case study 1

Contact-induced cell death of cancer cells by macrophages



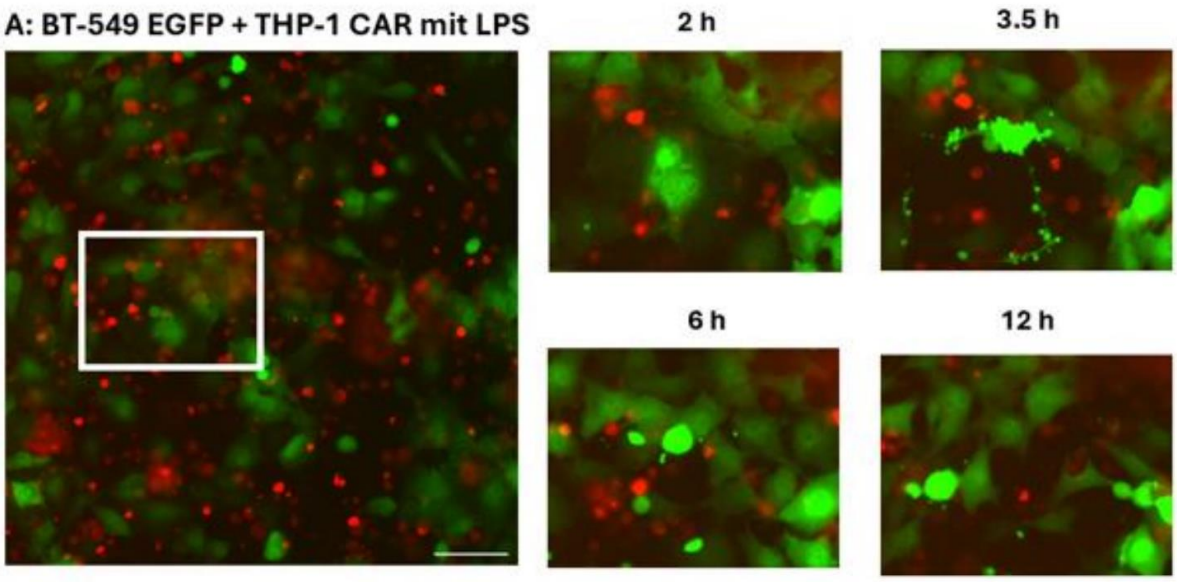
Current immunotherapeutic approaches explore the use of genetically modified immune cells such as, for example, T-cells or macrophages, i.e. professional phagocytes, equipped with an chimeric antigen receptor (CAR). CAR – immune cells are specifically recognizing and binding tumor antigens, which results in immune-cell mediated tumor cell killing.

The aim of this experiment was to use live imaging to observe the cell death of cancer cells (BT-549 GFP) after the interaction of with CAR macrophages (CAR-THP-1) in a 2D co-culture system.

The 2D co-culture consisted of GFP expressing BT-549 cancer cells and THP-1 CAR macrophages cells, which had been labeled with CellTracker deep red, and cultured for 24 hours.

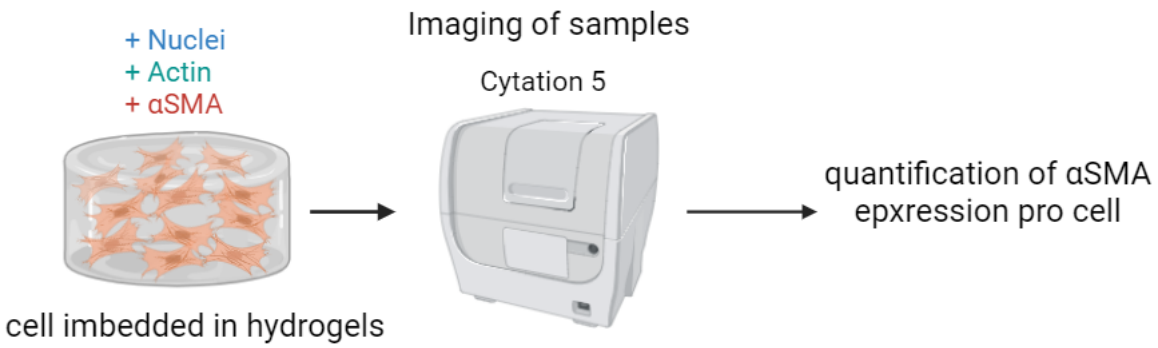
Cell death was detected through vesicle formation or cell fragmentation. Four different time points were selected for each condition to document the first observed cell death of a cancer cell. An example of the cell fragmentation is shown in the figure below, after 3.5h cell death occurred.

Your task is to quantitatively analyze the occurrence of cell death by analyzing the cell morphology/cell blebbing/apoptosis/fragmentation and bursting of the cancer green cells over time.



Case study 2

Quantification of the αSMA expression in patient derived cells cultured in a 3D environment



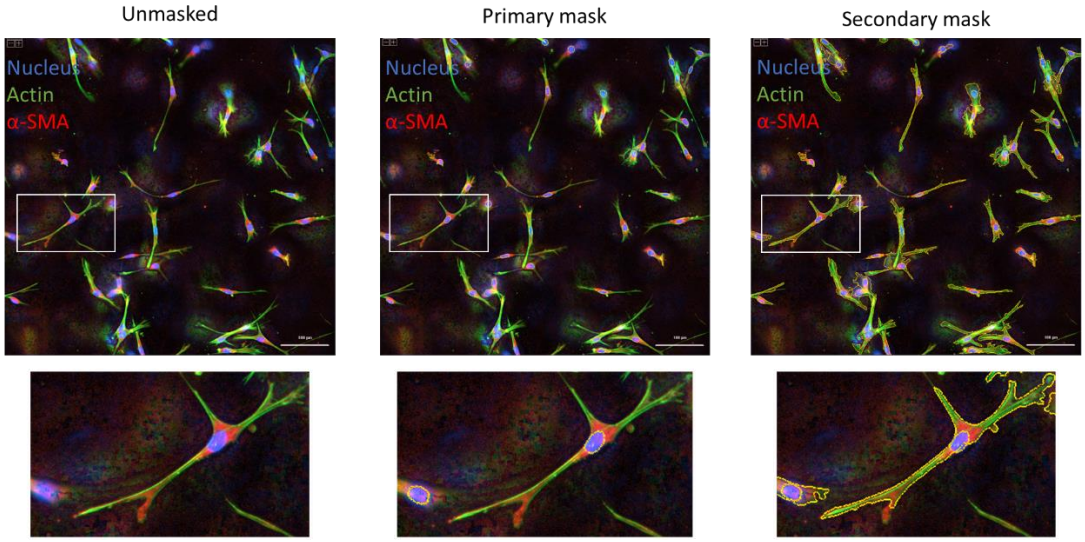
Fibrotic disease, characterized by accumulation of connective tissue, is involved in almost 50% of deaths in the western world. Yet, efficacious anti-fibrotic therapies are still missing. We are therefore working on the set up of a drug screen to identify novel anti-fibrotic therapies. To this end, primary fibroblasts, isolated from biopsies of patients suffering from implant associated fibrosis, are cultured in a 3D hydrogel pattern, and characterized.

During fibrosis, fibroblasts (connective tissue cells) are activated and differentiate into so-called myofibroblasts, contractile cells, producing large amounts of extracellular matrix components, which accumulate and replace healthy tissue over time. One of the hallmarks of myofibroblasts is a α-smooth muscle actin (αSMA) expression.

The aim of the experiment was to quantify the expression of αSMA in human patient-derived cells. Cells were cultured in 3D hydrogels for 24 hours with or without TGF-β1, a growth factor promoting myofibroblast differentiation. It is known that the presence of the inflammatory cytokine TGF-β1 enhances the expression of αSMA. To evaluate its expression in different patient-derived cells, antibody staining of αSMA was performed, along with additional staining of cell nuclei and actin filaments.

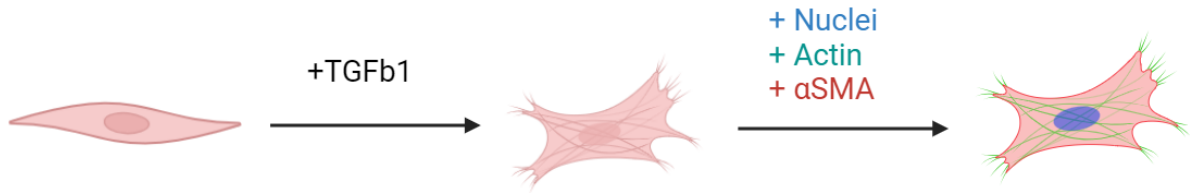
In the figure below, such a cell staining is shown, and αSMA expression was quantified by first segmenting the cell nuclei with a primary mask, then expanding the mask to include the actin filaments to define the cell edges. Finally, αSMA was quantified by measuring the amount of red fluorescence in each cell.

Your task is to perform cell segmentation and quantify the levels of αSMA/cell in the different settings.


Unmasked
Primary mask
Secondary mask
Nucleus
Actin
α-SMA
Nucleus
Actin
α-SMA
Nucleus
Actin
α-SMA

Case study 3

Differentiation of fibroblast to myofibroblast via cell morphology changes



Beyond changes in protein expression, fibroblast differentiation into myofibroblasts is associated with changes in cellular morphology. While normal fibroblasts show an elongated, spindle-shaped morphology, myofibroblasts develop protrusions and have a star-shaped morphology.

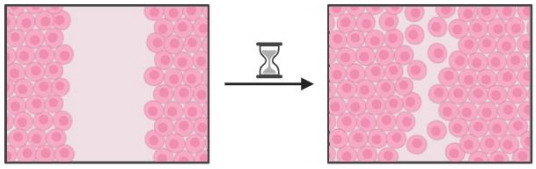
The aim of the experiment was to evaluate changes in the morphology of different patient-derived cells. Cell morphology refers to the shape of cells, which can include round, elongated, spindle-shaped, or irregular forms.

Cells were cultured in 3D hydrogels and incubated with or without TGF-β1 for 24 hours. To visualize the cell boundaries, cells were co-stained with Hoechst for nuclei and Alexa 488-phalloidin for actin filaments. Additionally, antibody staining for α-smooth muscle actin (αSMA) was performed, which is relevant for only for case study 2. The presence of TGF-β1 is known to alter the morphology of patient-derived cells, shifting them from an elongated shape (commonly observed in fibroblasts) to a star shape (typically seen in myofibroblasts), as shown in the figure above.

Similar to case study 2, in order to analyze single cell shape and identify myofibroblast cells, segmentation of the cell boundaries must be performed. Morphological descriptors, such as shape factor ($4\pi A/P^2$, where A is the area of the object and P is the perimeter), aspect ratio (long axis length/short axis length), and eccentricity, will need to be assessed under the different treatment conditions.

Case study 4

Effect of antifibrotic drug TOP N53 on the migratory properties of patient derived cells



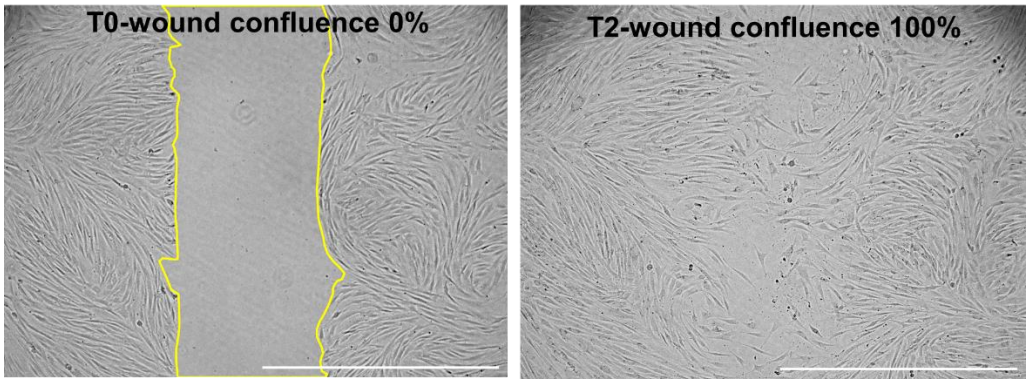
Scratch assay to investigate the migration behaviour of patient derived cells

Myofibroblasts have previously been demonstrated to be less migratory than fibroblast cells.

The aim of this experiment was therefore to evaluate the migratory properties of patient-derived cells using a scratch assay. Cells were seeded in a 2D culture and incubated with TGF-β1 for 48 hours and antifibrotic drug TOP N53. After incubation, a scratch was mechanically inserted in the center of the cell layer using an automated scratch device. Bright-field images of the cells were captured over a 24-hour period.

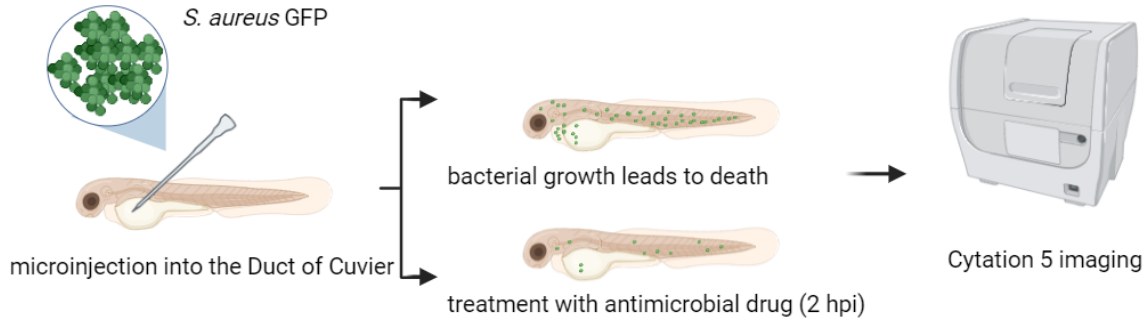
TGF-β1 is known to reduce the migratory properties of cells. Therefore, we aimed to assess the migration of patient-derived cells in the presence and absence of TGF-β1. In addition, TOP N53 is a clinical drug used for wound healing. We aim also to assess its antifibrotic properties.

Your task will be to measure the rate at which the scratch closes over time as the cells migrate.



Case study 5

Zebrafish embryo model for the screening of antimicrobial drugs

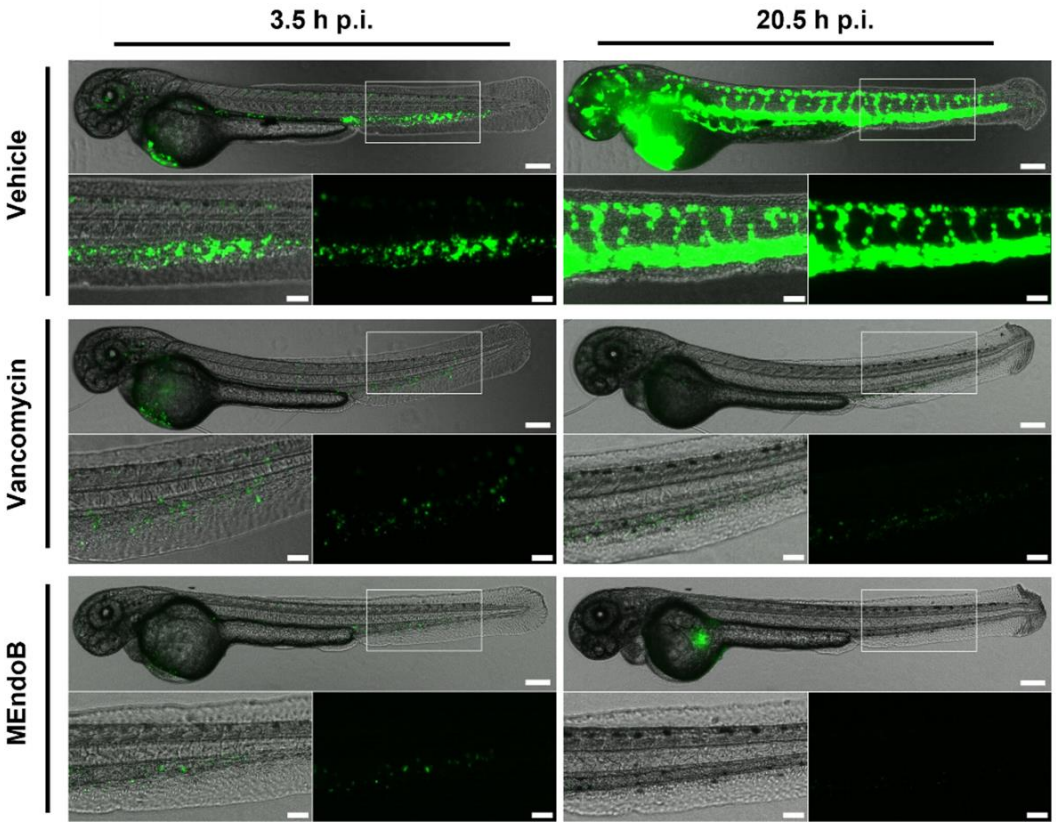


Zebrafish embryos are an excellent model for performing in vivo pharmacology experiments. Not only are they transparent and cost-effective, but they also share many homologous genes with humans. Using a whole organism model provides more reliable and comprehensive results.

Zebrafish larvae have been demonstrated to be a very suitable model of bacterial infections., allowing to in vivo characterize the efficacy of novel antimicrobial drug compounds. The aim of this experiment was to evaluate the effectiveness of antimicrobial drugs against Staphylococcus aureus using zebrafish embryos.

Zebrafish embryos (48 hours post-fertilization) were systemically injected with GFP-expressing *S. aureus*. Two hours after bacterial injection, the embryos were systemically injected with an antimicrobial drug (for example Vancomycin or MEndoB). The embryos were imaged over time, and the fluorescence signal from the bacteria within the embryos was quantified.

Your task will be to quantify the GFP signal of the bacteria in the zebrafish embryo over time to assess if and to which extent the drug influences the bacteria growth.


3.5 h p.i.
20.5 h p.i.
Vehicle
Vancomycin
MEndoB