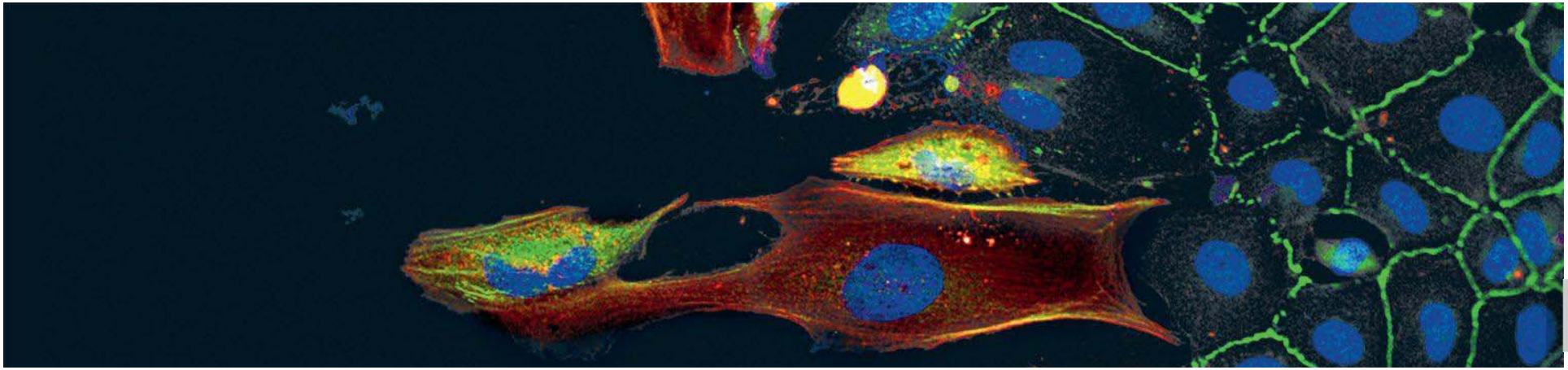
Cell atlas of the sick heart



Single cell techniques provide new insights at the cell level

Heart and vessels form a highly complex organ system in which extremely diverse cells have to work together correctly to provide all organs with blood. In past decades, heart biology placed its focus on whole tissues or cell isolates. Now, however, new technologies allow the tracing of a diversity of cell types and their individual responses to signals down to the level of proteins and genes. Researchers hope this will help them better support the regeneration of diseased hearts.

In an adult, endothelial cells, which line all blood vessels, cover the area of a soccer field. Endothelial cells work together with vascular muscle cells, which stabilise vessels and regulate pressure. Myocardial cells are in turn responsible for the contracting of the heart. These and other specialised cell types are integral to the dynamic interplay that maintains organ function.

The procedures used previously to study the diversity of cells and their regulations – especially in the case of diseased or older hearts – have a limited resolution so that relevant biological information can be lost. They are not capable of estimating the diversity of cell types and their individual responses to signals.

Newly developed procedures for analysing single cells now provide insights into the actual diversity of cells within the cardio-vascular system. As opposed to previous observation

How do individual cells react to risk factors?

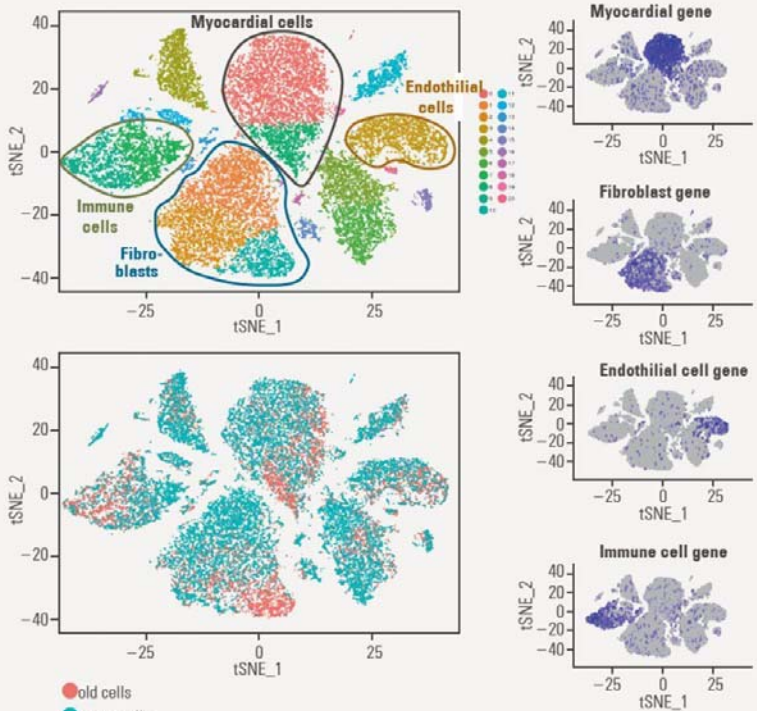
We can use these methods in disease models to first study how individual cells respond to risk factors or diseases. We want to find out, for example, whether all cells change at the same time, or if it is possibly only single cells or cell groups that damage neighbouring cells through incorrect contacts. It is particularly significant that these technologies can also be applied to small human tissue samples, such as biopsies, so that we can make an atlas of the diseased heart or vascular system. However, we first have to collect extensive data.

With regard to mice, matters are much further along: a cell atlas of a healthy mouse was recently published by the “Tabula Muris” Consortium: It comprises 100,000 cells of 20 organs and tissues. To create a human cell atlas, the Consortium “Human Cell Atlas” was founded in London in October 2016. Considering that an adult human consists of 100 trillion cells, it will still

with microscopy and flow cytometry, these procedures make it possible to simultaneously analyse a variety of active genes or proteins in individual cells (see box “Single cell technologies”). Approaches from artificial intelligence are used to bring all this data together and interpret it efficiently. With the help of machine learning procedures, scientists are able to discover signatures and classify cells with similar characteristics. In heart tissue, for example, endothelial cells, smooth muscle cells and myocardial cells, as well as other cell types can be differentiated and registered quantitatively according to their characteristics/expression patterns (Figure 1).

require a lot of work to reproduce this heterogeneity. With regard to some types of cells that occur in high numbers a small random sample is sufficient, but for rare cells (such as stem cells) or to determine complex changes in the course of disease, many cells have to be analysed. The Chan Zuckerberg Initiative by Facebook-founder Mark Zuckerberg and his wife, the paediatrician Priscilla Chan, funded the project with an additional $ 68 mil in June 2019.

1 Analysis of the gene expression of single cells in the young and old mouse heart



A The RNA of each individual cell from the young and old heart is decoded using single cell technologies, and a bioinformatics comparison is carried out for all cells. Using a machine learning procedure, the calls can be visualised two-dimensionally. Cells with very similar RNAs appear very close to each other in this analysis and form a cluster. Based on known, typical genes the cells can then be attribute to individual cell types (see example in B).

B Here, myocardial cells, fibroblasts, endothelial cells and immune cells can be seen. However, the individual cell populations also contain subgroups (such as the red and green cluster in the myocardial cell population in image A).

C Division of the cells into the old (red) and young heart (turquoise). The shift indicates that individual cell populations change in the aging heart. By determining the RNAs found in new cell populations, new signatures or signal paths can be discovered.

Data: Julian Wagner and David John in collaboration with Sascha Sauer, Berlin.

In a nutshell

- Modern single cell technologies make it possible to analyse a variety of active genes or proteins in individual cells.
- Researchers are currently gathering data from diseased heart and vascular tissue to find out whether and how individual cell groups change. There are initial findings regarding scar formation after heart attacks, atherosclerosis and the development, renewal and aging of heart cells.
- Special machine learning techniques are necessary in order to analyse the large volumes of data.

Defective cell communication following a heart attack

Our work group works together with Professor Andreas Zeiher, cardiologist, and Professor Thomas Walter, heart surgeon, with the support of the Dr Robert Schwiete Foundation to elucidate single cell biology in the diseased and aged human heart. In particular, we try to understand how individual cell types change in patients with heart diseases using human blood and small tissue samples from heart operations. A central focus is the question of how a heart attack and the resulting scarring change the composition and communication of cells in the heart.

In particular, we hope to discover how important human vascular and myocardial cells change at the cell level. Are there perhaps rare, previously unknown populations of stem or precursor cells that can be discovered at the single cell level? How do inflammation cells in blood change in patients with cardio-vascular diseases and which changes do they cause after migration into the heart?

While human samples have to first be collected, we have already won insights from initial studies on mice about how age affects their hearts. We were able to show that a change of individual cell populations can be observed in old age (Fig 1). Subsequent bio-informatics analyses also showed a change in the genes that are responsible for the communication of the cells among each other. We were able to document a defect in communication in the old heart. In young hearts, the cells support each other mutually: cells of the connective tissue which

Atherosclerosis: New target structures for medications in sight

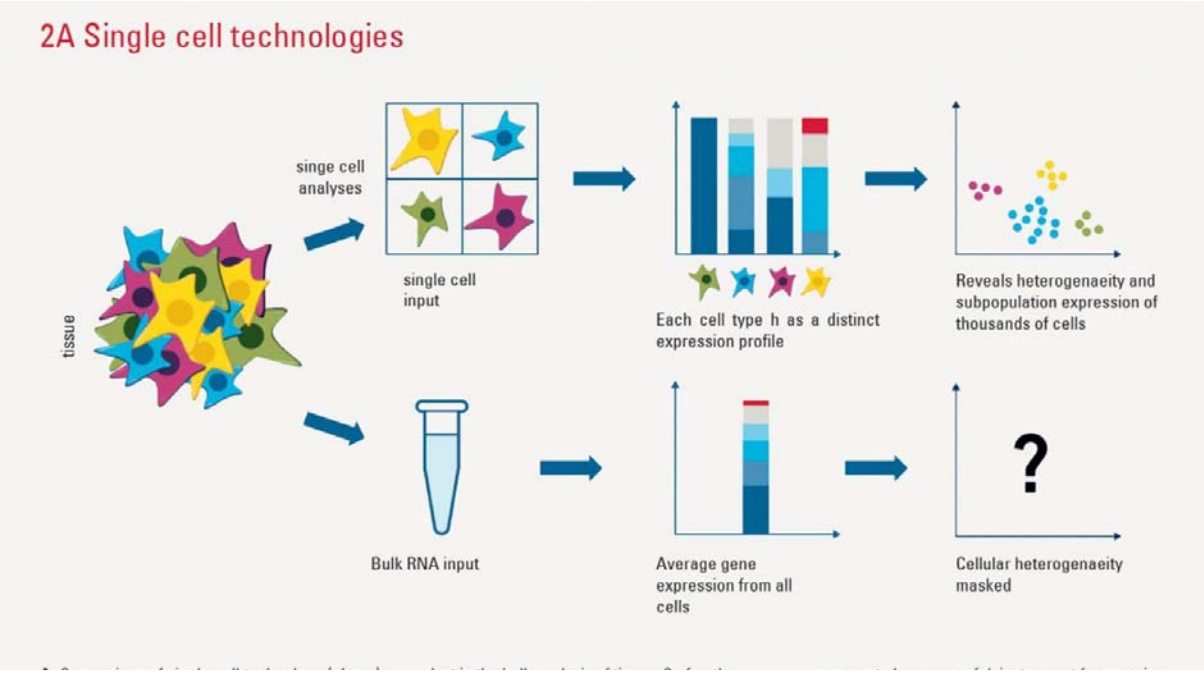
Initial studies using samples from experimental vascular disease models have also led to interesting results: Professor Nina Wettschureck, member of the “Cardio-Pulmonary Institute” and researcher at the Max-Planck Institute for Heart and Lung Research in Bad Nauheim, was able to demonstrate that chronic vascular inflammation leads to a particular activation of smooth muscle cells in the aorta, which in turn leads to a pathological change in blood vessels. The diseased cells differ from healthy cells by the expression of special genes, for example, various G-protein-coupled receptors. Since this type of receptor can easily be regulated by pharmacological substances, it is now being tested whether exclusively treating the diseased cells can halt the inflammation of the vascular system and possibly even the atherosclerosis.

Other researchers at the “Cardio-Pulmonary Institute” are attempting to use this new single cell technology for the discovery of rare cells such as stem cells and precursor cells. Professor Thomas Braun from the Max-Planck Institute for Heart and Lung Research in Bad Nauheim extensively characterised the cardiac precursor cells of mice in embryonic development. In addition to the analysis of gene expression, his group developed a procedure for assessing the epigenetic control in individual cells. In this way he was able to document various, previously unknown heart sub-populations. The reconstruction of the

support each other mutually: cells of the connective tissue which are located between the myocardial and vessel-forming cells, also known as fibroblast cells, secrete mediators that have a positive influence on vascular cells. In old age, the fibroblasts form different factors that have a negative effect on the vascular cells and which can lead to restricted blood circulation in the old heart.

development trajectories also show how multipotent stem cells are divided into different development branches before special genes cause the development directly into myocardial cells. By elucidating these epigenetic processes, Braun's team hopes to better understand the formation of myocardial cells, thereby possibly also discovering new approaches for the regeneration of the heart.

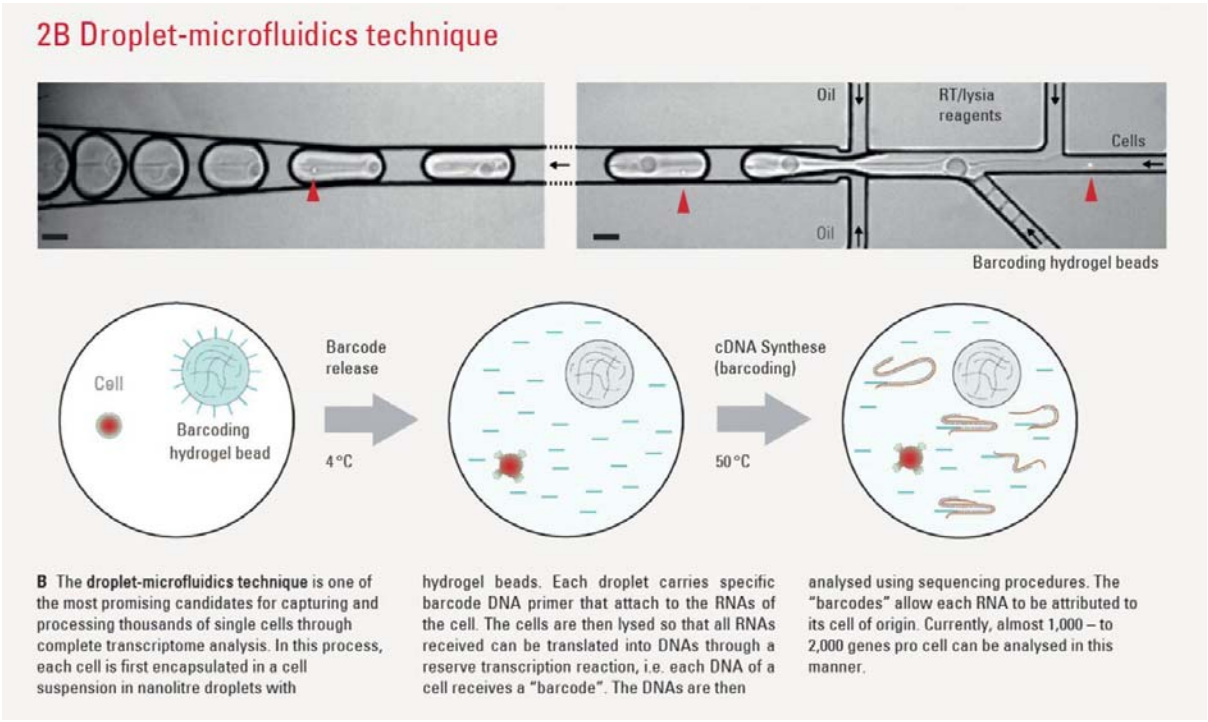
At the same time, the technology is being continually developed, so that more genes per cell can be analysed and proteins can be better identified. We hope that these new technologies contribute to a better understanding of cardio-vascular diseases, and that this will lead to the development of new therapeutic and diagnostic procedures.



Professor Stefanie Dimmeler is Speaker of the Excellence Cluster Cardio-Pulmonary Institute, shared by Goethe University, Giessen University, and the Max

A Comparison of single cell technology (above) with the bulk analysis of a tissue (below). Single cell technologies allows the determination of the signatures of individual cells on different molecular levels. This allows conclusions to be drawn regarding regulation that get completely lost in the bulk analysis of tissue. So far, the analysis of DNA and RNA and the first steps toward determining the epigenetic control and proteins have been successful. Technologically, **single cell RNA analysis** is the most developed and most widespread. In recent years, it has proven to be a powerful instrument for mapping the cellular heterogeneity of diseased and healthy tissues. High throughput methods are required in order to capture the variety of cells.

by Goethe University, Giessen University, and the Max Planck Institute for Heart and Lung Research in Bad Nauheim. The biologist heads the Institute for Cardiovascular Regeneration at Goethe University. Her main area of research is the elucidation of mechanisms of vascular and heart repair.



B The **droplet-microfluidics technique** is one of the most promising candidates for capturing and processing thousands of single cells through complete transcriptome analysis. In this process, each cell is first encapsulated in a cell suspension in nanolitre droplets with hydrogel beads. Each droplet carries specific barcode DNA primer that attach to the RNAs of the cell. The cells are then lysed so that all RNAs received can be translated into DNAs through a reserve transcription reaction, i.e. each DNA of a cell receives a "barcode". The DNAs are then analysed using sequencing procedures. The "barcodes" allow each RNA to be attributed to its cell of origin. Currently, almost 1,000 – to 2,000 genes pro cell can be analysed in this manner.

Dr. Wesley Abplanalp is an American physiologist who has been working on cardiovascular regeneration since February 2017. He is in charge of the Institute's single-cell analysis platform.

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