Spatial omics analysis of the cellular immune landscape and their infiltration routes in a minimally invasive mouse model of myocardial infarction

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Myocardial infarction (MI) is one of the leading causes of death worldwide, accounting for about 20% of deaths annually.

Infiltration of infarcted tissue by immune cells plays a crucial role during the acute phase of MI.

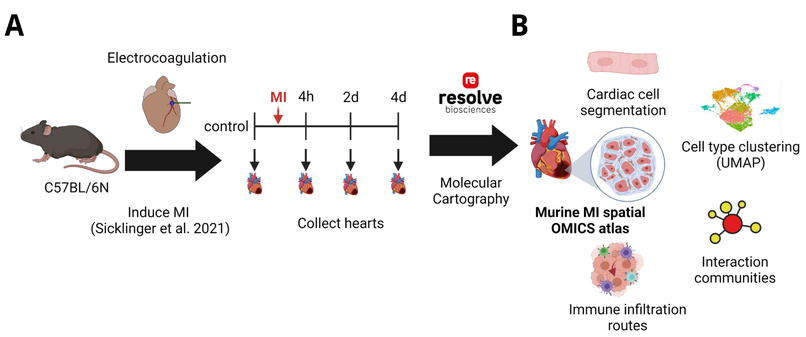
Highly multiplexed, spatial analysis using Molecular Cartography enables a novel perspective on immune cell infiltration in a spatiotemporal manner at unprecedented resolution.

Abstract

Myocardial infarction (MI), commonly known as a heart attack, results from lack of oxygen supply (ischaemia) to cells of the ventricular heart wall. Following an acute myocardial infarct, necrotic cells release soluble messenger molecules, such as Damage-associated molecular patterns (DAMPs) and pro-inflammatory cytokines and chemokines, leading to the attachment and invasion of the infarct zone by immune cells - specifically neutrophils and monocytes. While the general immune cell-types and signals implicated in the sterile inflammatory response to myocardial infarction have been extensively studied, the spatiotemporal infiltration routes into the infarct zone are less well understood. Here, we use a novel, minimally invasive method to induce MI in mice followed by highly-multiplexed in-situ sequencing of 100 transcripts to investigate the spatial dynamics of immune cell infiltration in the acute phase (4 hours , 2 days, 4 days) following a myocardial infarct. Our results reveal remodelling of the highly organized tissue architecture from healthy myocardium to a fibrotic scar via an early pro-inflammatory response followed by monocyte infiltration via the border zone, epicardial as well as the endocardial layer.

What are the spatio-temporal, cellular shifts occurring in the inflammatory region during acute murine myocardial infarction?

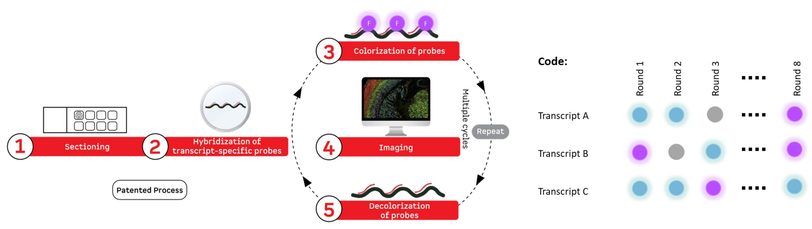
Experimental design



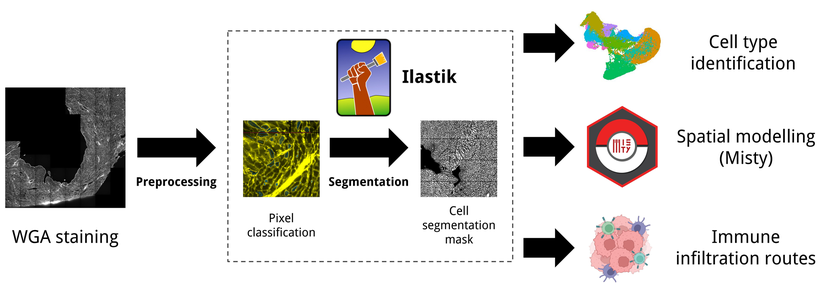
A) We used a novel, minimally invasive model established by Sicklinger et al (2021) to induce medium sized infarcts in 12 week old, female C57Bl/6 mice in duplicate at 4 different time points (control, 4h, 2d, 4d). This novel technique allows us to create highly reproducible infarcts with minimal mortality and off-target effects in comparison to traditional induction methods.

B) Using Molecular Cartography, we investigated infarcted hearts samples at high resolution. We performed cardiac cell segmentation using a custom semantic segmentation model in Ilastik (Berg et al. 2019). Using spatially resolved, single-cell level expression data, we assigned major cardiac cell types, reconstructed interaction communities and identified different immune infiltration routes in the infarcted tissue.

Methods



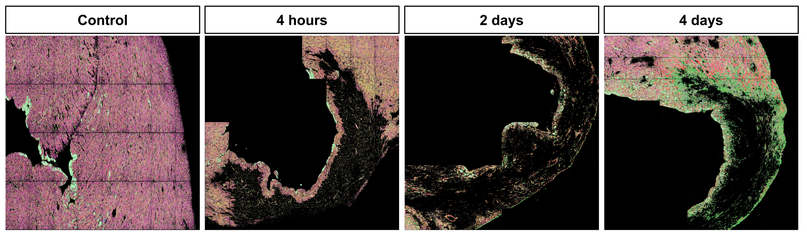
Resolve Bioscience Molecular Cartography method was used to measure 100 transcripts at subcellular resolution, that were selected based on literature knowledge and existing scRNA-seq data (see references).



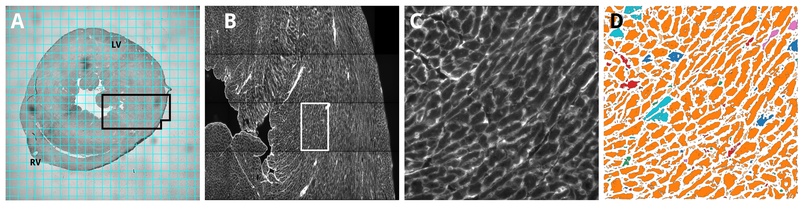
We used WGA staining to visualize cardiac cell membranes in left ventricular sections. A semantic segmentation model was built using Ilastik and the masks were used to assign spots to cells. The transcript data assigned to single-cells was subsequently used to identify major cell types, perform spatial modelling via Misty (Tanevski et al. 2022) and calculate infiltration dynamics of cardiac macrophages.

Results

Molecular cartography of 100 transcripts in infarcted left ventricular mouse tissues

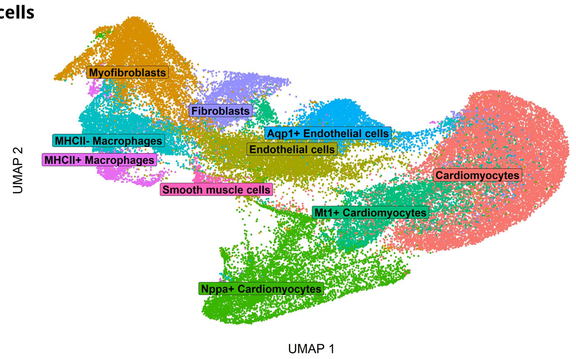


ROI selection and cell segmentation of cardiac tissue sections based on WGA staining



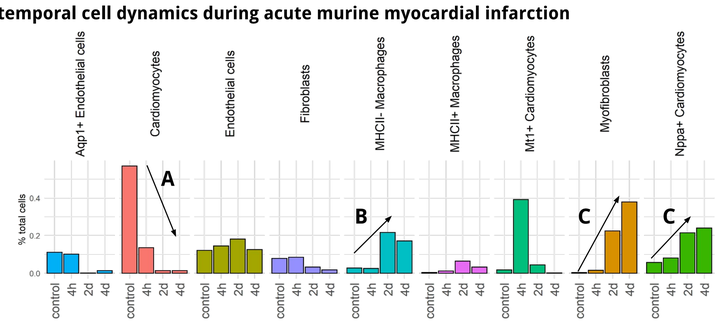
Whole tissue brightfield image with region for Molecular Cartography highlighted (A), WGA staining (B, C) and zoom-in of cell segmentation mask (D) with assigned cell types from murine ventricular tissue in a control sample. RV = right ventricle, LV = left-ventricle.

Cell type identification based on Molecular Cartography transcript spots assigned to segmented cells



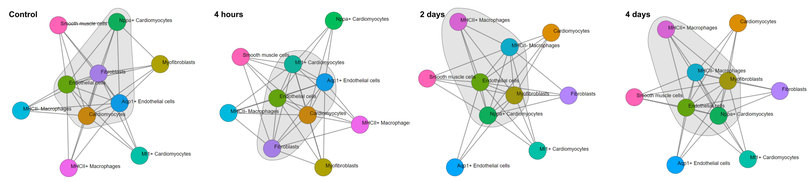
UMAP embedding of 53212 cells from 8 molecular cartography heart samples across four sampled time points (control, 4h, 2d, 4d) in duplicate .

Spatiotemporal cell dynamics during acute murine myocardial infarction



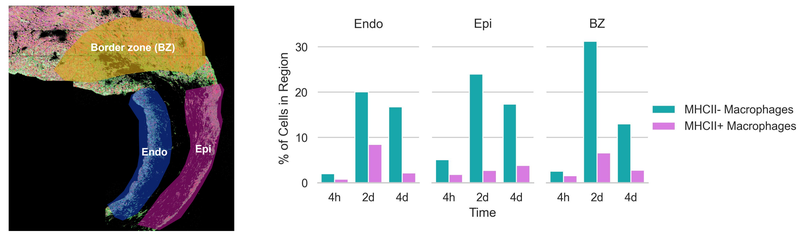
Fraction of cardiac cell types during acute MI per time point. **A)** Cardiomyocytes (CM) are abundant in healthy control tissue but quickly die and stress adjacent CMs (Mt1+ Cardiomyocytes) at 4h after an infarct. **B)** Around day 2 and day 4, the scar tissue gets infiltrated by macrophages, reflected by their increase in relative abundance. **C)** Myofibroblasts establish the extracellular matrix inside and around the scar tissue and become the predominant cell type by day 4. Finally, cardiomyocytes surrounding the scar tissue increasingly start expressing the hormone natriuretic peptide A (Nppa+) over time.

Dynamic tissue architecture changes from healthy myocardium via an early inflammatory response towards a fibrotic scar as captured by Misty



Network community plots from Misty, representing changes in the local cardiac microenvironment before and during acute myocardial infarction.

Infiltration of the infarct scar by macrophage populations occurs via the border zone (BZ), the epicardial (Epi) and the endocardial regions (Endo)



Fraction of macrophage cells in the border zone, epicardial and endocardial regions. Cell counts were normalized to total cell count in the respective zone. MHCII positive (+) and negative (-) macrophages utilize all three regions to invade the scar tissue following an acute myocardial infarct with an increase in relative abundance at day 2 and day 4, reflecting known temporal infiltration dynamics.

Outlook

We will validate our transcript based findings with antibody-based methods to further delineate potentially distinct monocyte subpopulations in the different anatomic infarct zones.

We want to use this dataset as a starting point to explore clinically relevant models. We are particularly interested in the effects of sex, age and currently applied, standard of care pharmacological treatments and their impact on the cellular microenvironment and tissue architecture during acute MI.

Funding, Acknowledgments & Resources

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