Investigating the Effect of Cardiac Macrophages on Excitation-Contraction Coupling in Heart Failure

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Background

Heart Failure (HF): The heart is unable to pump around the body blood sufficiently.

Hallmark: Cardiac *remodelling* - The heart's structure and function change in response to injury/underlying conditions. **Cardiac Macrophages** (cMacs) contribute to this process.

cMacs:

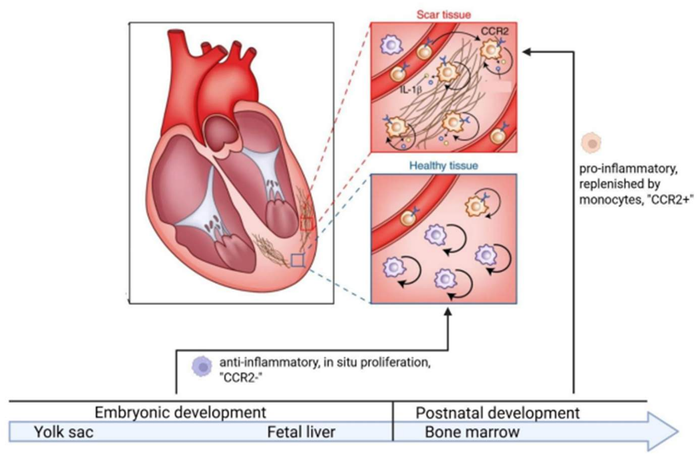

Figure 1. Simplified overview of cMac ontogeny and implications in heart disease. During embryonic development cMacs are either derived from yolk-sac macrophages or fetal liver monocytes. CCR2 is a chemokine receptor expressed only in cMacs replenished by monocytes. Small yellow and blue dots represent cytokines either secreted by CCR2+ macrophages or by other cell types. Adapted from (1)

Embryonic cMacs "CCR2-"
- anti-inflammatory, reparative, self replenish

In animal models a reduction in number causes:
↓ LV systolic function
↑exaggerated ventricular remodelling
↑ mortality in cardiomyopathy
(2-4)

Recruited cMacs "CCR2+"
- pro-inflammatory, maintained through monocyte infiltration

In animal models both reduction and increase in number *can* cause pathology

Reduction in number:
↑ LV systolic function
Increase in number:
↑ fibrosis, remodelling, HF severity (2-4)

Disease mechanisms are poorly understood → **excitation-contraction coupling** (ECC) modulation?

ECC links electrical activation of a cardiomyocyte to calcium release and sarcomere shortening, converting an action potential into a mechanical heartbeat.

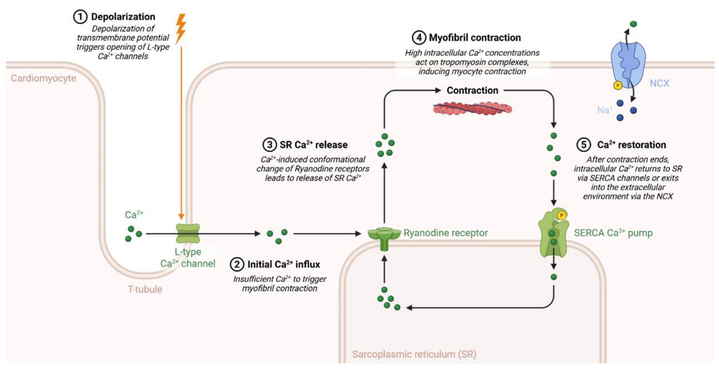

Figure 2. Schematic of calcium-induced calcium release in ECC. Depolarisation (1), triggers Ca2+ influx (2 and 3), which causes contraction (4). Then, Ca2+ levels are restored (5).

Hypothesis

cMacs affect ECC of failing cardiomyocytes.

Aim 1: To assess the effect of cMac and monocyte-derived macrophage number and activation state on ECC under physiological, diseased, and disease-simulating in vitro conditions in human living myocardial slices (LMS) from donor and failing hearts.

The Model - Human Living Myocardial Slices

Living myocardial slices (LMS) are thin (300-400 µm), viable sections of heart tissue that preserve the native cellular architecture and multicellular interactions of the myocardium.

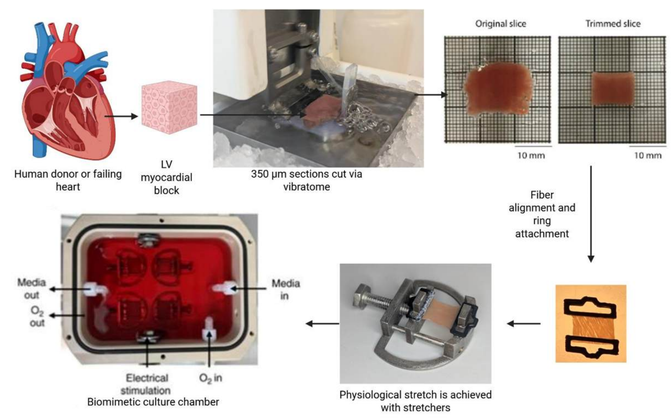

Figure 3. Overview of generation of LMS and culture setup.

NC3Rs

LMS have been optimised for many animal models including rats and pigs. This project uses *human tissue only* - **Replacement** has been achieved.

Human Tissue is available through

NHS Blood and Transplant

INOAR program - Increasing the Number of Organs Available for Research

Between July 2024 and October 2025 we accepted 53 human donor hearts. Failing hearts (e.g. hypertrophic cardiomyopathy, HCM) from patients receiving a heart transplant were sent from Harefield hospital.

Experimental Setup

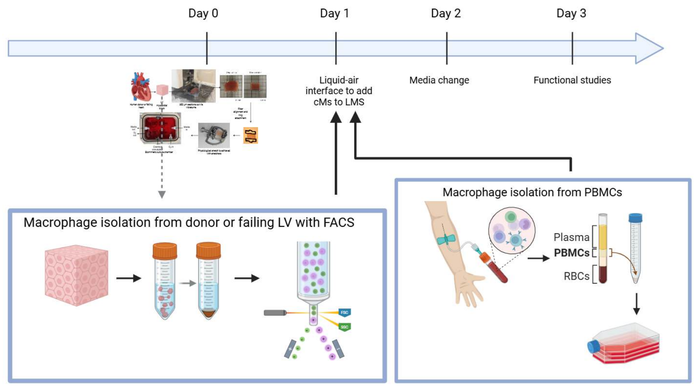

Figure 4. Overview of experimental process. Day 0: Heart receival and LMS generation and culture. Day 1: Liquid-air interface to add macrophages to LMS. Day 2: Media change. Day 3: Functional studies.

Results

cMacs in their native tissue environment

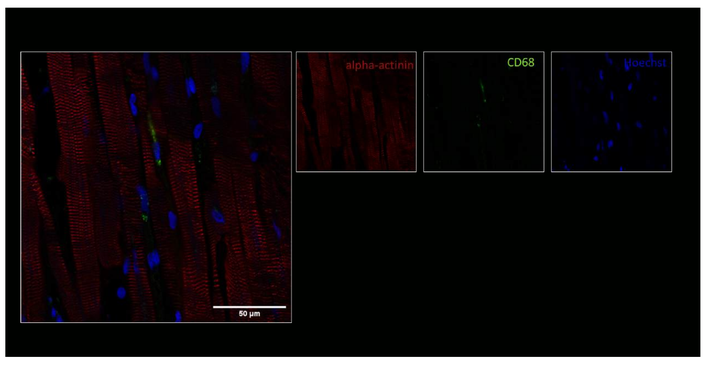

Figure 5. Immunofluorescent image of cMacs (CD68, green) in D0 LMS. 63x.

Results

cMacs were successfully isolated from donor myocardium

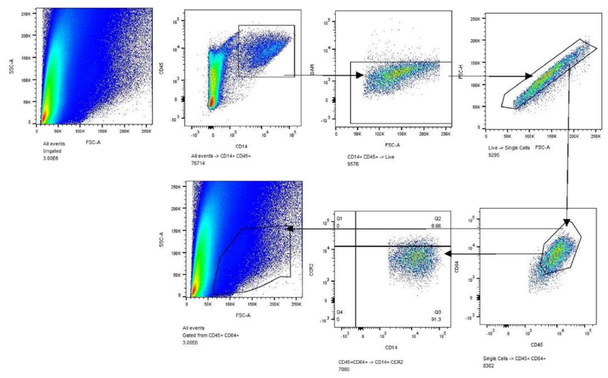

Figure 6. FACS sorting strategy. After excluding debris by positively selecting for CD14+ events, and dead cell and doublet exclusion, CD45+ CD64+ CD14+ CCR2+/- cells are sorted.

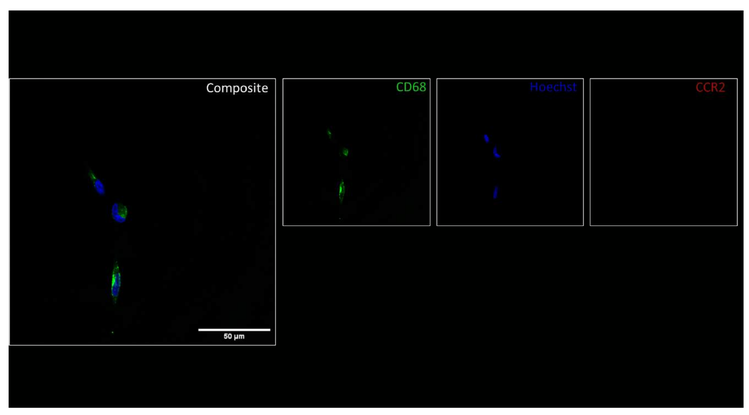

Figure 7. Representative immunofluorescent image of sorted cMacs. After sorting cMacs were seeded into culture chambers and cultured for 3 days. 63x.

Peripheral blood monocyte-derived macrophages may affect active force and time to peak of human LMS

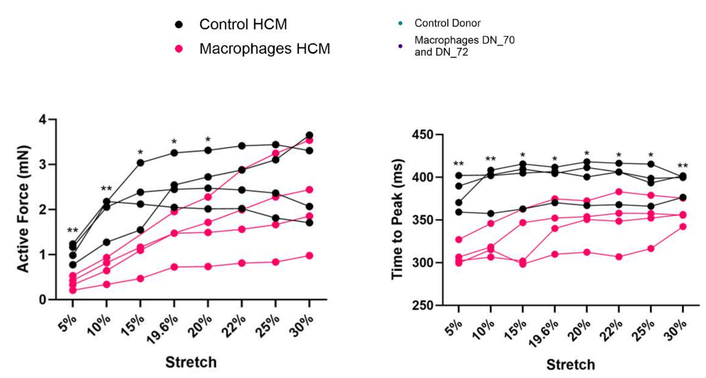

Figure 8. Active force and time to peak of LMS from failing heart (HCM) after 72 hours in culture. $1x10^6$ macrophages/ LMS. N = 1, n = 4 per experimental group. Statistical testing: Shapiro-Wilk for normality and a 2-way-ANOVA. $P < 0.05$ is indicated with *, $p < 0.01$ with **.

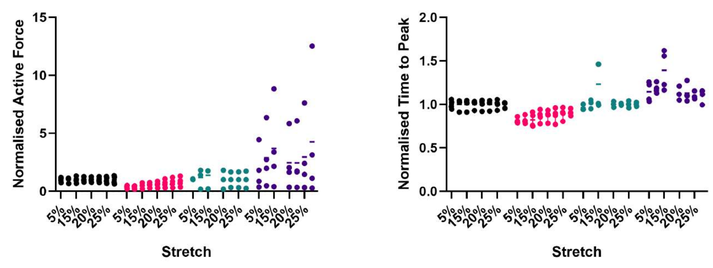

Figure 9. Normalised active force and time to peak after 72 hours in culture. $1x10^6$ macrophages/ LMS. N=3 (1 HCM, 2 Donor hearts), n=4. No statistical testing due to low n numbers.

Conclusion

- Human LMS are a powerful alternative to animal LMS to study the effect of cMacs on cardiomyocyte function
- Macrophages might affect active force and time to peak of human LMS

References

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