Characterization of Human sEVs Derived from Living Myocardial Slices Undergoing Myocardial Hypoxia-Reoxygenation Injury

Fani Koutentaki[1], Linjie Zheng[1], Samuel Reitemeier[1], Danika Hayman[1], Richard Kelwick[2], Paul S Freemont[2], Cesare M Terracciano[1]
[1]National Heart and Lung Institute, Imperial College London | [2]Section of Structural and Synthetic Biology, Department of Infectious Disease, Imperial College London, London
Email: fani.koutentaki20@imperial.ac.uk

1. Background

1.1. Hypoxia-reoxygenation injury (HRI)

Coronary Heart Disease (CHD) remains the leading cause of death globally.[1]

8 million people are living with cardiovascular disease

Every 8 minutes someone in the UK dies from coronary heart disease

£12 billion is the total annual cost of cardiovascular disease in the UK

Myocardial **ischemia reperfusion injury (IRI)** is caused by the occlusion of a coronary artery and leads to restriction of blood supply to the left ventricle (LV), followed by its restoration.[2]

An element of IRI is **hypoxia-reoxygenation injury (HRI)** related to the restriction of O_2 supply to the LV, followed by its restoration.

The timely restoration of blood flow following ischemia is essential; however, it is itself associated with myocardial damage[3]

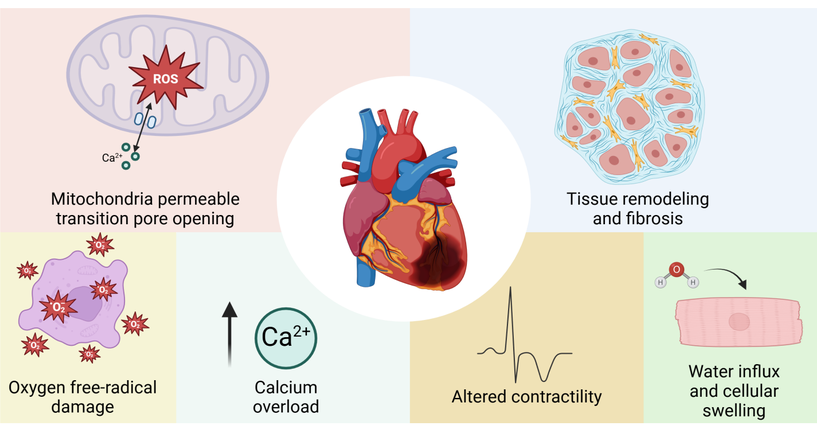

Figure 1 | Effects of IRI[3]

IRI effects can lead to contraction impairment, arrhythmia, and eventually heart failure[2-3].

1.2. Small extracellular vesicles (sEVs)

sEVs are lipid bilayer-delimited particles of **endocytic origin**, range in **size 30-200nm**, and are **produced by all cell types.** They contain **bioactive cargoes** – DNA, lipids, proteins, miRNA – contributing to **intercellular communication**[4].

1.3. sEVs in myocardial disease

In cardiac disease:
✓sEVs increase in circulation
✓sEVs cargoes change in response to stimuli

Myocardial functional, structural, and biochemical changes

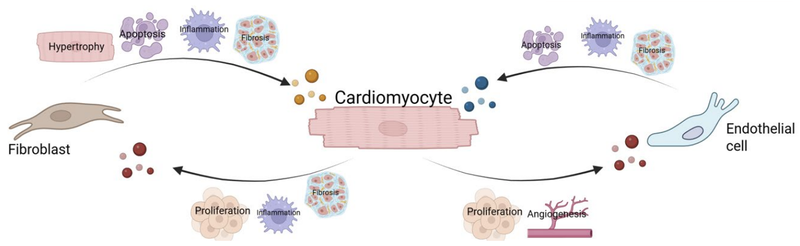

Figure 2 | Pathways affected by sEV-mediated cardiac cell communication[5]

Unclear mechanistic role of the increased sEV secretion in the IRI pathogenesis and disease progression due to lack of experimental models

1.4. Human living myocardial slice model (LMS)

The LMS model is an ultra-thin tissue layer derived from adult human donor hearts. It is the model of intermediate complexity, as it maintains the multicellular and matrix components of the myocardium[6].

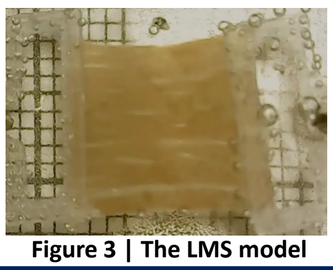

Figure 3 | The LMS model

2. Hypothesis

Hypoxia-reoxygenation of LMS induces the secretion of sEVs with defined characteristics that have a regulatory role in the establishment, prevention and/or progression of myocardial injury

3. Plan of investigation

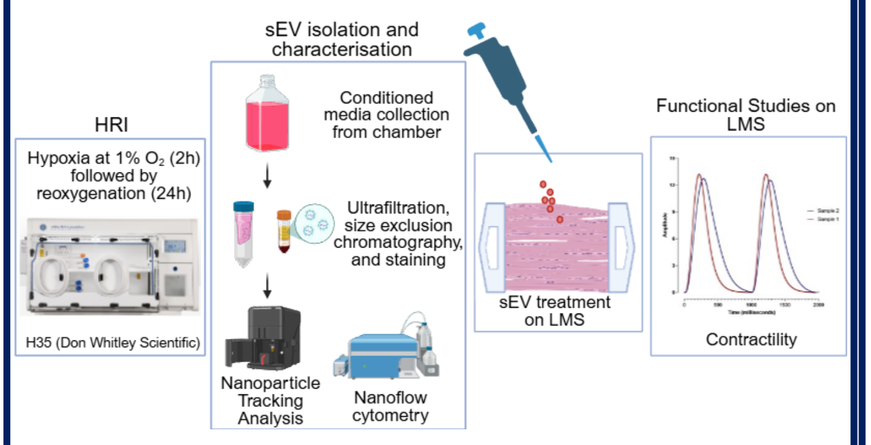

Figure 4 | Overview of the plan of investigation

Application of the 3Rs in this research

Human hearts are available from the Increasing the Number of Organs Available for Research (INOAR) initiative of the UK NHS Blood and Transplant (NHSBT). In this context, hearts that are not transplantable are retrieved for research.

The retrieval can be performed by Cardiothoracic (CT) or Abdominal (Abdo) Surgical Retrieval Teams. The CT Team perfuses the heart with cardioplegia solution, while the Abdo Team does not. The cardioplegia solution provides key components to the heart during the process, preserving it for research.

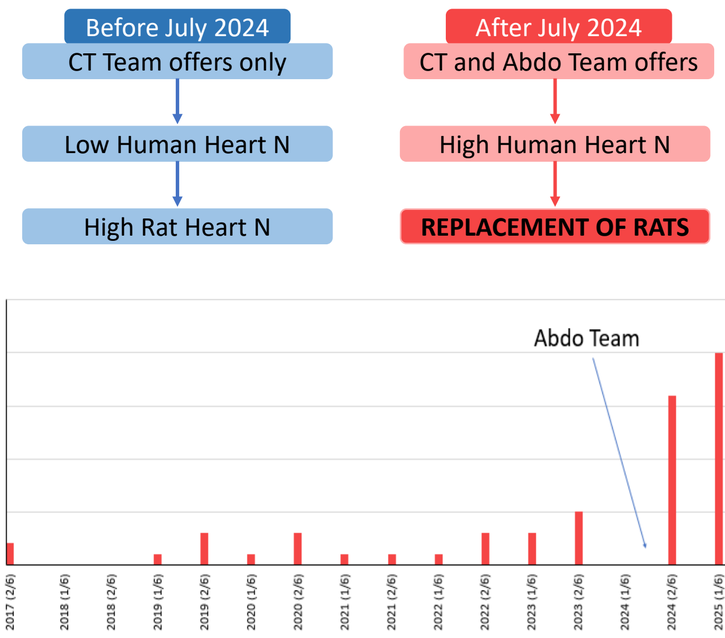

Figure 5 | Human hearts accepted for research following the application of 3Rs. *X*-axis: Time (6 months), *y*-axis: Number of accepted hearts. The number of hearts accepted increases from July 2024.

4. Methods

4.1. Human LMS preparation and culture

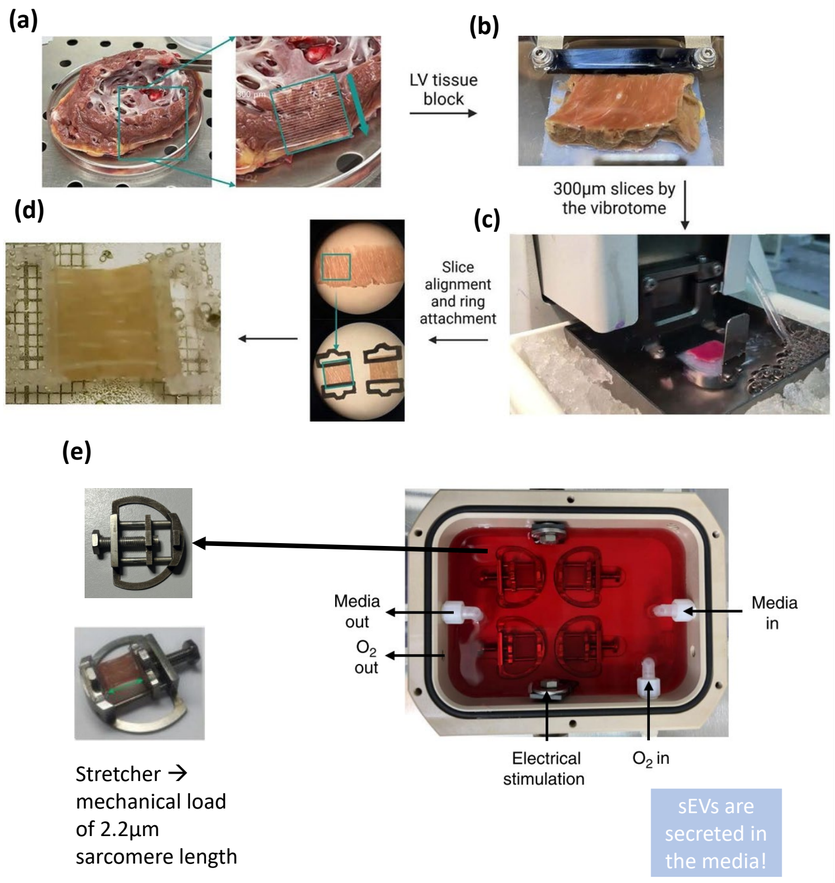

Figure 6 | Preparation of the LMS model[6]. (a) Human donor hearts provided from the NHS Blood and Transplant INOAR program are dissected to obtain an LV block. **(b, c)** The block is sliced in 300µm slices by the vibratome. **(d)** The slices are aligned for the attachment of rings, to obtain the final LMS. **(e)** LMSs are placed on stretchers that provide mechanical load. They are cultured in a biomimetic chamber in 37°C supplied with 100% O_2, electrical stimulation, and media circulation to recapitulate physiological conditions.

4.2. Hypoxia-reoxygenation injury

Following **24 hours in culture**, the chamber Is transferred to the **H35** set at **1% O_2, 5% CO_2, and 94% N_2** at 37°C and in **de-oxygenated media.** After **2h of hypoxia**, the chamber is transferred back to **reoxygenation** condition in **fresh oxygenated media**[6].

4.3. sEV application on liquid-air interface of LMSs

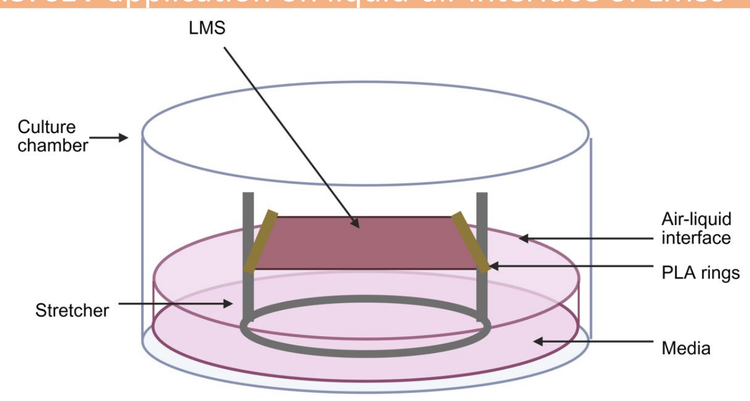

Figure 7 | Setup of the air-liquid interface of sEVs treatment on LMS[7]. The top surface of the LMS is exposed to air. The sEVs are applied on the surface for absorption by the LMS. Electrical stimulation, media circulation, and oxygenation are interrupted. Following absorption, media is added to achieve the desired volume and electrical stimulation, media circulation, and oxygenation are resumed.

4.4. Functional Studies

Functional studies are performed using the **Force Transducer apparatus**. LMS **contractility** is recorded under electrical stimulation at **multiple stretches**[6].

5. Results

5.1. The active force and time to peak of contraction is significantly reduced following HRI

The **active force** of HRI LMSs is **significantly reduced (p<0.0001), specifically at 20%, 22%, 25%, and 30% stretch** compared to control LMSs (Fig. 8a). **The time to peak** of contraction of HRI LMSs is also significantly decreased (p=0.0002) (Fig. 8b).

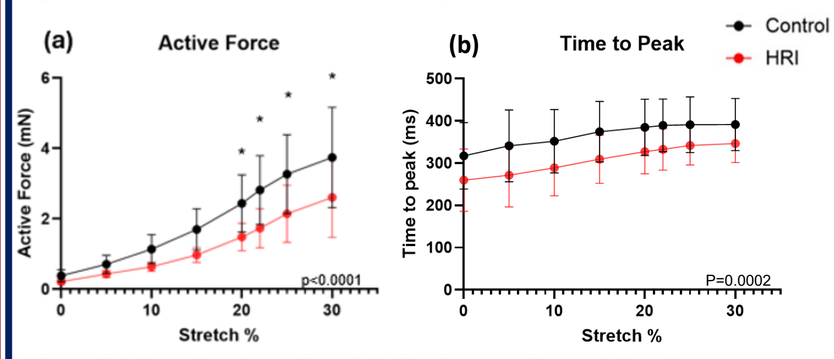

Figure 8 | Functional studies of LMSs. Contractility and kinetics parameters following 48h in culture. **(a)** Active force (mN), **(b)** Time to peak (ms). *X*-axis: Stretch %, *y*-axis: contractility or kinetics parameter. Data are shown as mean ± SD. Significance was tested with ordinary 2-way ANOVA with Tukey's post hoc test or with unpaired t-test. N=5 human donor hearts, with n=3-4 LMSs for each condition per heart. Significance is indicated with * above data points, where * < 0.05.

5.2. The secretion of sEVs is increased following HRI

The secretion of **30-200nm sized sEVs** is **significantly increased following HRI (TP3)** compared to control LMSs (Fig. 9a). The **total size distribution of sEVs in TP3** indicates that the **peak sEV concentration** is achieved within the **size range of 100-200nm** (Fig. 9b). The sEVs indicate positive **expression of CD63** (sEV marker) (Fig. 9c-d).

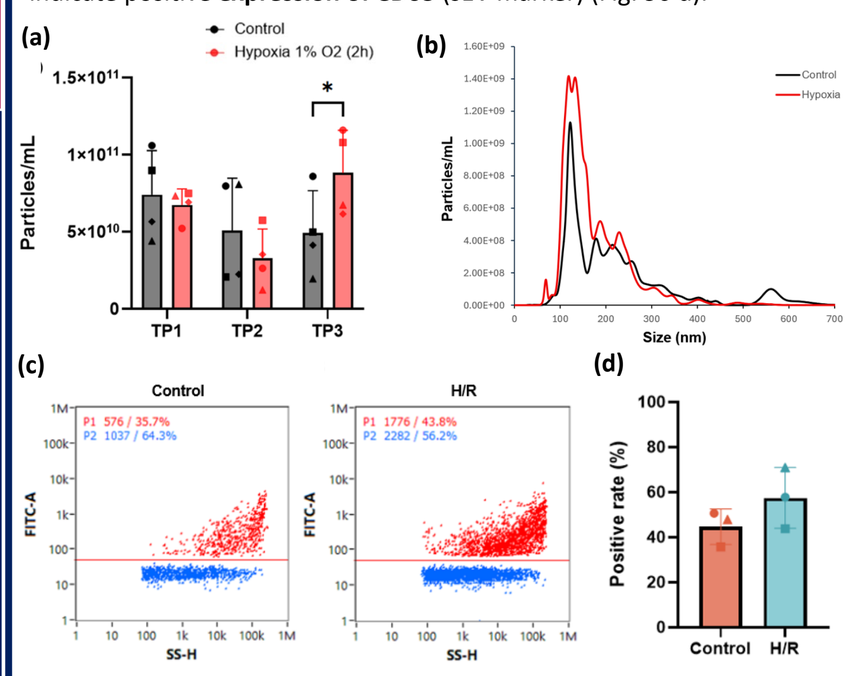

Figure 9 | Characterization of sEVs. (a) Concentration of sEVs across the three timepoints of HRI. *X-axis:* TPs %, *y-axis*: particles/mL. **(b)** Size distribution of sEVs at TP3. *X-axis:* size (nm), *y-axis*: particles/mL. Data are shown as mean ± SD. Significance was tested with ordinary 2-way ANOVA with Tukey's post hoc test. N=4 human donor hearts. **(c)** Representative output from nanoflow cytometry for CD63 expression. **(d)** Proportion of CD63-positive cells. Significance is indicated with * above data points. *TP1=* Proportion of CD63-positive sEVs. *before hypoxia; TP2= after 2h 1% O_2 hypoxia; TP3= after reoxygenation.*

5.3. The time to peak of contraction is significantly increased following the application of HRI sEVs on healthy LMSs

The **time to peak of contraction** of healthy LMSs is **significantly increased** (p=0.0453) following the application of HRI sEVs (Fig. 10b).

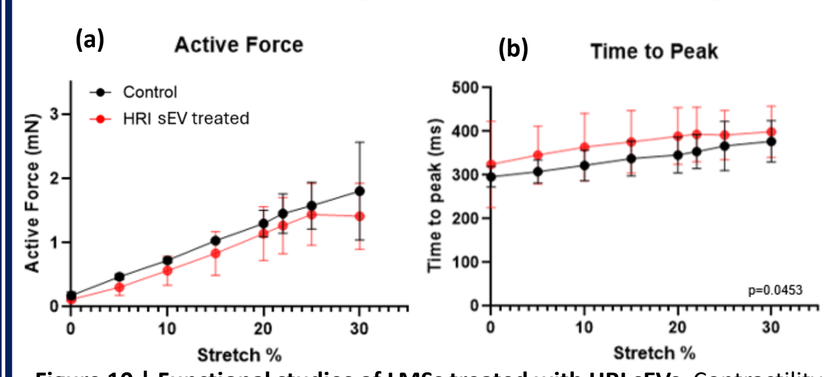

Figure 10 | Functional studies of LMSs treated with HRI sEVs. Contractility and kinetics parameters following 48h in culture. **(a)** Active force (mN), **(b)** Time to peak (ms). *X*-axis: Stretch %, *y*-axis: contractility or kinetics parameter. Statistics are performed as previously. N=3 human donor hearts, with n=3-4 LMSs for each condition per heart.

6. Summary and Future Directions

1. LMSs exposed to 1% O_2 hypoxia for 2 hours followed by 24 hours reoxygenation exhibit a **reduction in active force and time to peak parameters of contraction** and **an increase in sEV secretion**.
2. The application of HRI sEVs on healthy LMSs induces a **prolonged contraction** phenotype.
3. Future directions include the characterization of the **structural effects and Ca^{2+} activity** of HRI on the LMSs and the investigation of **sEV marker expression** and their **change in content**.

7. Conclusion

Hypoxia-reoxygenation injury increases the release of sEVs. Replacing rat hearts with human tissue enables scientifically meaningful and ethically responsible research.

References: [1] British Heart Foundation. UK Cardiovascular Disease Factsheet (2026). Available online at: chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.bhf.org.uk/-/media/files/for-professionals/research/heart-statistics/bhf-cvd-statistics-uk-factsheet-jan26.pdf?rev=26534e1487094dbd806277891baef112 (Accessed January 15, 2026). [2] Parkhomenko, O.M., Amosova, K.M., Andriyevska, S.O., Golobordko, B.I., Zhurba, S. V., et al. (2021) Universal definition of myocardial infarction. *Ukrainian Journal of Cardiology*. 28 (6). doi:10.31928/1608-635X-2021.6.4168. [3] Yellon, D.M. & Hausenloy, D.J. (2007) Myocardial Reperfusion Injury. *New England Journal of Medicine*. 357 (11). doi:10.1056/nejmra071667. [4] Shanmuganathan, M., Vughs, J., Noseda, M. & Emanueli, C. (2018) Exosomes: Basic biology and technological advancements suggesting their potential as ischemic heart disease therapeutics. *Frontiers in Physiology*. 9 (NOV). doi:10.3389/fphys.2018.01159. [5] Emanueli, C., Shearn, A.I.U., Laftah, A., Fiorentino, F., Reeves, B.C., Beltrami, C., Mumford, A., Clayton, A., Gurney, M., Shantikumar, S. & Angelini, G.D. (2016) Coronary artery-bypass-graft surgery increases the plasma concentration of exosomes carrying a cargo of cardiac microRNAs: An example of exosome trafficking out of the human heart with potential for cardiac biomarker discovery. *PLoS ONE*. 11 (4). doi:10.1371/journal.pone.0154274. [6] Watson, S.A., Scigliano, M., Bardi, I., Ascione, R., Terracciano, C.M. & Perbellini, F. (2017) Preparation of viable adult ventricular myocardial slices from large and small mammals. *Nature Protocols*. 12 (12). doi:10.1038/nprot.2017.139. [7] Koutentaki F, Nicastro L, Kelwick RJR, Freemont PS and Terracciano CM (2025) Investigating the mechanisms of small extracellular vesicles in cardiovascular disease using the living myocardial slice platform. *Front. Cardiovasc. Med.* 12:1697099. doi: 10.3389/fcvm.2025.1697099.