Investigating cardiovascular roles of non-vesicular extracellular nanoparticles

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Background

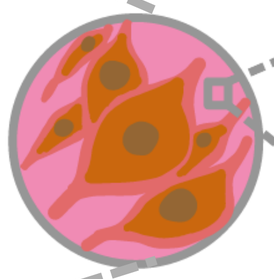
Ischaemic Heart Disease (IHD)

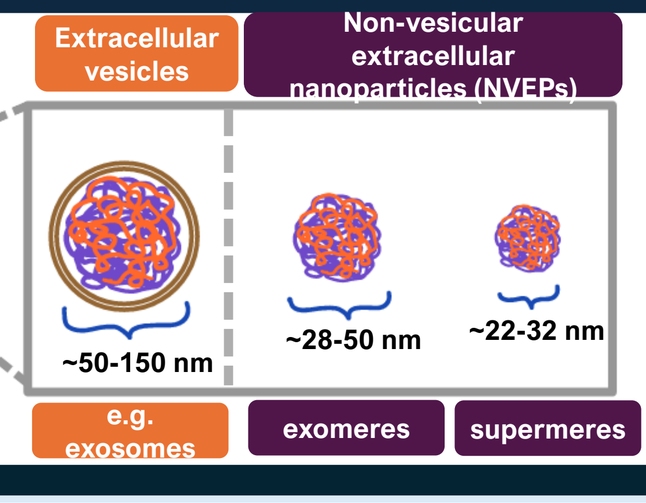
Most prevalent type of cardiovascular disease→ affects ~126 million people globally (1).

- Main cause: atherosclerosis → lack of myocardial blood supply→ tissue death, myocardial infarction, arrhythmia, heart failure. (2)
- Current treatments: starts with non-surgical interventions (lifestyle changes, medication) and then surgical interventions with increased severity (coronary artery bypass graft (CABG), percutaneous coronary intervention (PCI)).
- Need for therapies that address damage to tissues such as fibrosis and blood vessel reduction.
- Hence, this project is investigating MSC-derived non-vesicular extracellular nanoparticles for this purpose.

Mesenchymal Stem Cells (MSCs) and secretome in IHD (3,4)

- Pro-angiogenic
- Anti-fibrotic
- Cardiomyocyte proliferation
- Immunomodulatory effects





Hypothesis and Aims

Hypothesis: MSCs NVEPs→ cardiac protection and regeneration potential ↑ angiogenesis, ↓fibrosis and ↑ cardiomyocyte viability

Aim 1: Isolation and characterisation of MSC and myocardial slice-derived NVEPs.

Aim 2: Analyse effects of MSC NVEPs or related cargo on myocardial slices and cells

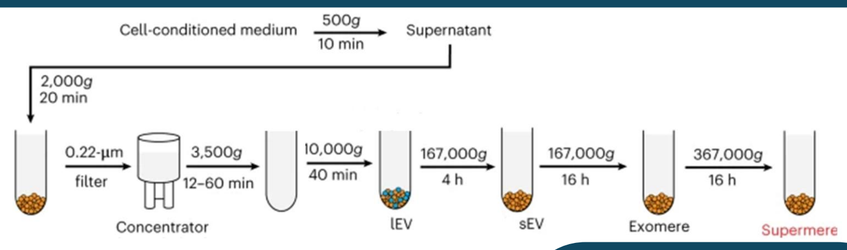
Aim 3: To establish an in silico electromechanical mathematical model simulating the myocardial slice

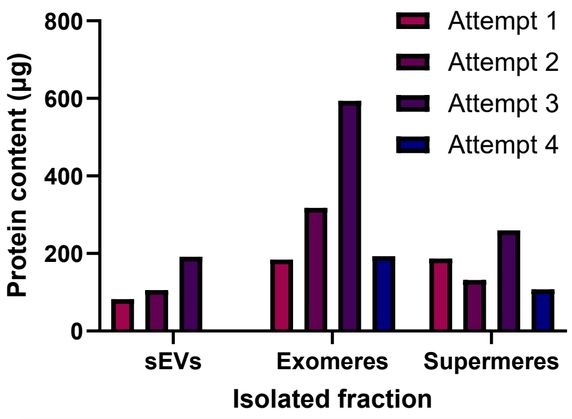
Plan of Investigation and Progress

NVEPs

Isolation method

~70 million MSCs (~15 x T175s) are grown and kept in serum-depleted media for 48 hours prior to conditioned media collection. Subsequently subjected to filtration, concentration and a series of differential ultracentrifugation steps to isolate large EVs, small EVs, exomeres and supermeres (5,6). Schematic adapted from (5).





Protein yield

Isolated fractions are analysed by Pierce BCA (protein quantification assay) between each batch.

→ High variability in protein yield between different fractions and different batches.

→ Improved handling of protocol→ improved yield.

Characterisation

- Negative-stain (2% uranyl acetate) transmission electron microscopy was used with supermeres as a test case (shown below).
- Dynamic Light Scattering was also attempted → exomeres and supermeres not detected.

Challenges

- Heterogeneity in protein yield.
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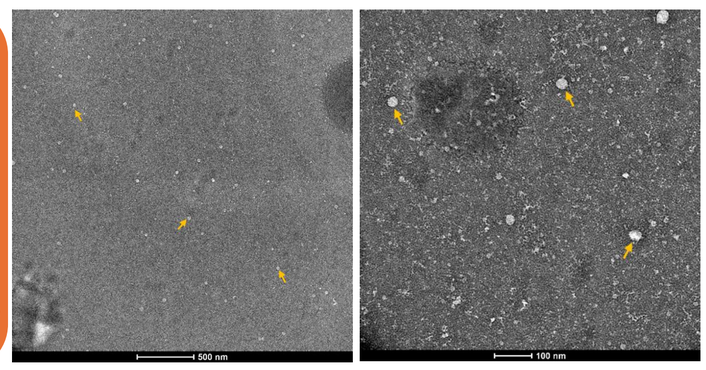
Difficult/inconclusive characterisation as current automatic label-free methods (NTA) cannot resolve them.



Next Steps

Samples (n=3) will be sent for proteomics (Aim 1 to identify cargo) to identify MSC- exomeres/supermere markers. Currently, one batch is undergoing a pilot run to assess methodology.

This project focuses on the **Replacement** objective of the **3Rs** and as such I will be using **human-derived models** as outlined below.

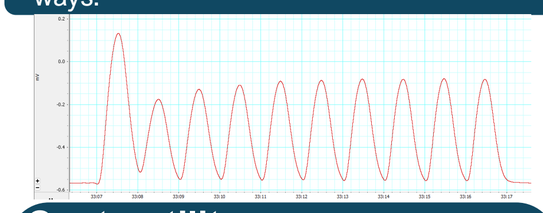
Cells

- Human Cardiomyocytes (AC16s)
- Endothelial cells (HUVECs)

- Treated and untreated cells will be assessed for viability, proliferation and angiogenesis (HUVECs)
- Experiments with AC16 are being conducted currently using a WST-1 assay. 40 ug/ml was used in initial experiment. Next step → dose response curve to find optimal dose.

Myocardial Slice

Untreated and treated (with NVEPs or relevant proteomic cargo) myocardial slices will be assessed in three ways:

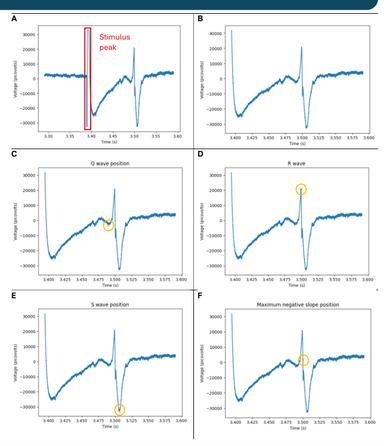


Contractility

- Contractility will be measured via a tissue bath apparatus with a force transducer (trace shown above). This set-up is connected to a Micropace EP which stimulates the slice at 1Hz with a pacing current of 20-25mA with a 10ms duration.

MEA

Micro-electrode array (MEA) measurements (shown on the right) to extract time-domain (e.g. activation and end-point times) and frequency-domain features.

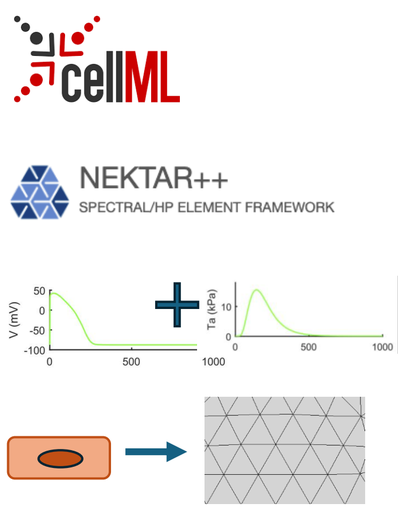


Immunostaining

Ki-67 (proliferation), collagen I and vimentin (fibrosis), isolectin-B4 (angiogenesis/microvascular rarefaction)

In silico electromechanical slice model

1. Identification of existing ventricular electromechanical cell models/ electrophysiological model and contractility model that can be combined.
2. Convert code from electrophysiological (EP) model and transfer into Nektar++
3. Extend EP model implementation to include contractility-related equations to establish electromechanical coupling, confirm biological relevance and perform optimisation of parameters, if necessary.
4. Translate from a single-cell scale to a 2D tissue level by creating a "fluid-like" mesh (homogenisation) with nodes connecting the single cells, allowing the simulation of electrical conduction and overall tension across a "tissue" of cardiomyocytes.
5. Validate *in silico* response with data obtained from myocardial slices and their response with pharmacological agents with known effects e.g. lidocaine, carbonoxelone, dopamine.



Next Steps

Currently troubleshooting conversion in step 2 for repeated pacing.

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