Mesenchymal Stem Cells for the Treatment of Myocardial Infarction-Induced Ventricular Dysfunction.

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Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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James David Richardson

December 2013
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Personal Bibliography

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Presentations & Prizes arising from the Thesis:

- Cardiac Society (CSANZ) 2012 Oral Presentations:
  - “Immediate Mesenchymal Stem Cell Therapy Provides Greater Attenuation of Myocardial Injury Than Deferred Treatment in Rats After Acute Myocardial Infarction.”
  - “Sequential Mesenchymal Stem Cell Interventions Produce Greater Myocardial Repair Than Solitary Treatment in Rats After Acute Myocardial Infarction”

- Cardiac Society (CSANZ) 2012 Poster Presentations:
  - “Prospectively Isolated, Hypoxic-Preconditioned Mesenchymal Stem Cells Significantly Attenuate Myocardial Infarction-Induced Ventricular Dysfunction In Rats”.
  - “Cardiac Magnetic Resonance, Transthoracic and Transoesophageal Echocardiography: A Comparison of In Vivo Ventricular Function Assessment in Rats”.
  - “Assessment of Regional Myocardial Function in Rats using 1.5T Cardiac Magnetic Resonance Imaging”

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- Genesis Research Award 2012 (Winner)

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# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>αMEM</td>
<td>Alpha modification of Eagle's medium</td>
</tr>
<tr>
<td>ESV</td>
<td>End-systolic volume</td>
</tr>
<tr>
<td>µg</td>
<td>Microgram</td>
</tr>
<tr>
<td>FACS</td>
<td>Fluorescence-activated cell sorting</td>
</tr>
<tr>
<td>µL</td>
<td>Microlitre</td>
</tr>
<tr>
<td>FCS</td>
<td>Foetal calf serum</td>
</tr>
<tr>
<td>µm</td>
<td>Micrometre</td>
</tr>
<tr>
<td>FITC</td>
<td>Fluorescein isothiocyanate</td>
</tr>
<tr>
<td>µM</td>
<td>Micromolar</td>
</tr>
<tr>
<td>FS</td>
<td>Fractional Shortening</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>GFP</td>
<td>Green fluorescent protein</td>
</tr>
<tr>
<td>BM</td>
<td>Bone marrow</td>
</tr>
<tr>
<td>HBSS</td>
<td>Hanks’ balanced salt solution</td>
</tr>
<tr>
<td>BMMNC</td>
<td>Bone marrow mononuclear cells</td>
</tr>
<tr>
<td>HGF</td>
<td>Hepatocyte growth factor</td>
</tr>
<tr>
<td>CD</td>
<td>Cluster of differentiation</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary deoxyribonucleic acid</td>
</tr>
<tr>
<td>IGF</td>
<td>Insulin-like growth factor</td>
</tr>
<tr>
<td>CFU-F</td>
<td>Colony forming units-fibroblast</td>
</tr>
<tr>
<td>IHD</td>
<td>Ischaemic heart disease</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>CMR</td>
<td>Cardiac magnetic resonance</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>CXCL12</td>
<td>Stromal cell-derived factor 1 (SDF-1)</td>
</tr>
<tr>
<td>LV</td>
<td>Left ventricle (or left ventricular)</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s modification of Eagle’s medium</td>
</tr>
<tr>
<td>MACS</td>
<td>Magnetic-activated cell sorting</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulphoxide</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>MI</td>
<td>Myocardial Infarction</td>
</tr>
<tr>
<td>EC</td>
<td>Endothelial cells</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>EDD</td>
<td>End-diastolic dimension</td>
</tr>
<tr>
<td>MPC</td>
<td>Mesenchymal precursor cells</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>MSC</td>
<td>Mesenchymal stromal/stem cells</td>
</tr>
<tr>
<td>EDV</td>
<td>End-diastolic volume</td>
</tr>
<tr>
<td>n</td>
<td>Sample number</td>
</tr>
<tr>
<td>EF</td>
<td>Ejection Fraction</td>
</tr>
<tr>
<td>TGF</td>
<td>Transforming growth factor</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>EPC</td>
<td>Endothelial progenitor cells</td>
</tr>
<tr>
<td>v/v</td>
<td>Volume per volume</td>
</tr>
<tr>
<td>ESD</td>
<td>End-systolic dimension</td>
</tr>
<tr>
<td>w/v</td>
<td>Weight per volume</td>
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Thesis Abstract

Despite current treatment options, cardiac failure after myocardial infarction (MI) is associated with significant morbidity and mortality so highlighting a compelling clinical need for novel therapeutic approaches. Based on promising pre-clinical data, stem cell therapy has been suggested as a possible therapeutic strategy. Early studies largely utilised autologous bone marrow cells with only modest benefits observed in clinical trials. Of the alternative candidate cell types evaluated, mesenchymal stromal/stem cells (MSCs) have shown promise, however their clinical application for mainstream cardiovascular use is currently hindered by several important limitations. Consequently, this has prompted intense efforts to advance the therapeutic properties of MSCs through cell optimisation strategies.

Allogeneic sources of MSC appear to hold several important advantages over autologous bone marrow/BM mononuclear cells (BMMNC); (1) MSC can be derived from young, healthy donors thereby enhancing the absolute yield and functional biology of MSCs; (2) The cell product could be prepared well ahead of time, so making very early MSC treatment feasible, e.g. after primary percutaneous intervention, when myocardium remains viable; (3) MSC could be optimised to potentially advance their therapeutic efficacy.

The studies described in this thesis utilised all of the above features to address the primary aims of:

1. Reviewing the literature and writing a review regarding the optimisation of the cardiovascular therapeutic properties of MSC.

2. Develop an allogeneic MSC population optimised by the novel combination of prospective-isolation enrichment and hypoxic preconditioning. Furthermore, evaluate the in vivo function of optimised MSC compared to conventional plastic-adherent isolation of MSC (PA-MSC).
3. Develop a reliable non-invasive assessment of rat ventricular function using 1.5T cardiac magnetic resonance and evaluate this modality against conventional methods (transthoracic echocardiography) and novel modalities in rats (transoesophageal echocardiography).

4. Explore the impact of the timing of MSC intervention and cell dose after MI, now that immediate cell intervention is feasible clinically and these factors have not previously been investigated.

5. Explore the potential benefits of immediate and deferred MSC treatment after MI, two very different time points – a novel concept.

An allogeneic source of MPCs was derived from donor rat bone marrow. In contrast to conventional plastic-adherent isolation of MSC, an enriched and optimised MSC population prepared by prospective isolation of immature MPCs (via a CD45 immunodepletion step) and hypoxic preconditioning was established. In cell-based experiments, optimised MSC were compared to same-donor plastic-adherence isolated MSC and demonstrated superior in-vitro differentiation and colony forming capacity than PA-MSC.

To evaluate the effects of MSC treatment after MI in rats, highly accurate and reproducible imaging techniques are required. Cardiac magnetic resonance (CMR) is widely regarded as the gold standard modality, however the use of standard 1.5T “clinical” MR scanners in rodents has only been achieved by a handful of investigators worldwide and none have used contemporary MR techniques. CMR was then evaluated against conventional imaging modalities (transthoracic echocardiography) and novel methods in rats (transoesophageal echocardiography).

Allogeneic MSC permits immediate treatment, previously impossible with autologous stem cells, therefore this potentially important variable (timing) was assessed. Myocardial infarction was induced by ligation of the left anterior descending artery in rats. Optimised
MSC were then injected into the myocardium either immediately after MI or one week later, at one of two cell doses. This study provided an innovative comparison of these clinically relevant time points and demonstrated value at both times. Furthermore, greater efficacy was observed with immediate treatment, which displayed high sensitivity to MSC dose, with benefits largely localised to the infarct territory. Deferred treatment, though less effective, was not dose dependant and primarily influenced non-infarct myocardium.

Given the disparate, yet beneficial effects, of immediate and deferred MSC intervention the benefit of combining MSC treatment at both time points was investigated. Again, this was undertaken in the rat model of MI, with CMR determination of ventricular function. This novel study showed clinically relevant improvements in LV function and confirmed the differential distribution of MSC repair according to timing of cell intervention.

In summary, the studies described in this thesis provide new evidence outlining the merits of prospective isolation and hypoxic preconditioning of MSC. Furthermore they demonstrate the reparative effects of these cells and provide novel insights into the significance of timing of MSC intervention on efficacy and mode/distribution of effect, which can be further augmented through treatment both time points after MI.