the injured myocardium signals splenic-monocyte mobilisation remains poorly understood. Recent work shows extracellular vesicles (EV, which carry proteins, microRNA/mRNA) are a means of rapid cell-to-cell communication, which, combined with knowledge of their composition and propensity to be taken up by other cells, suggests a possible role in signalling. Here we show that acute MI results in a net increase in circulating endothelial cell (EC)-EV that induce splenic monocyte motility *in vivo*.

Methods MI was induced in mice. EV were isolated by ultracentrifugation and analysed for size/number by Nanoparticle Tracking Analysis, western blot (EV-markers: Alix, TSG101, CD69, CD9 and Hsp70) and ELISA for EC markers (CD31, ICAM-1, P-selectin, E-selectin and VCAM-1). Primary cardiac mouse-EC (CM-EC) were used to produce EV *in-vitro* and were labelled fluorescently (PKH67) and transfected with cell-miR39-3p (*C.elegans*). EV were either tail vein injected into wild-type or CD68^{GFP+} naïve mice and/or exposed to macrophages (RAW 264.7) *in-vitro*.

Results Acutely (24 hours) after MI there was a significant increase in circulating EV in mice ($7.6 \pm 1.7 \times 10^8/\text{ml}$ vs $3.6 \pm 1.5 \times 10^8/\text{ml}$, control, $P < 0.01$) that later subsided ($4.6 \pm 1.8 \times 10^8/\text{ml}$ 4th day post-MI). Plasma EV displayed EC-surface markers, suggesting EC origin. Pro-inflammatory TNF- α ($3.0 \pm 0.1 \times 10^9/\text{ml}$ vs $1.5 \pm 0.1 \times 10^8/\text{ml}$ control, $P < 0.01$) significantly increased EV production in CM-ECs *in-vitro*, whereas 'anti-inflammatory' IL-4 ($1.3 \pm 0.01 \times 10^9/\text{ml}$) and IL-6 ($1.5 \pm 0.1 \times 10^9/\text{ml}$) had no effect. Inflammatory-EC-EV displayed significant enrichment of VCAM-1 ($P < 0.05$). *In-vitro* there is a time-dependent accumulation of labelled EV in macrophages. Inflammatory-EC-EV significantly enhanced macrophage chemotaxis to MCP-1 *in-vitro* (2.2 ± 0.2 AU EV treated vs 1.7 ± 0.2 AU untreated, $P < 0.01$), a response that was abolished by pre-incubating EC-EV with an anti-VCAM-1 antibody (1.8 ± 0.2 AU, $P < 0.05$). Inflammatory EV significantly induced alterations in mRNA of motility genes by reducing PLEXIN-B2 ($P < 0.001$) and increasing ITGB2 ($P < 0.001$) expression in macrophages. Injected labelled EC-EV accumulate in the splenic red-pulp and injected inflammatory-EC-EV induced significant splenic monocyte mobilisation and peripheral monocytosis *in-vivo* in CD68^{GFP+} naïve mice ($P < 0.05$).

In conclusion (1) *In vivo* EV are released acutely after MI; (2) Plasma-EV protein composition is consistent with endothelial cell origin. (3) Injected EV localise to the spleen and (4) mobilise splenic monocytes. (5) In culture, EV increase cell motility and (6) regulate genes that are important in motility. These findings are all suggestive of a role for EC-derived EV in monocyte activation after acute MI.

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CARDIOSPHERE-DERIVED CELL-SEEDED POROUS COLLAGEN SCAFFOLDS FOR CARDIAC REPAIR

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Irrespective of cell type, stem cell therapy to prevent heart failure following myocardial infarction is beset by low donor cell retention. Cell loss may be prevented by immobilisation within a supporting scaffold for application across the infarct,

but cells must remain viable while vasculature develops into the scaffold. Previously we have shown that cardiosphere derived cells (CDCs) can be cultured on porous collagen scaffolds (Chen *et al*, J. Tissue. Sci. Eng. 2012). Here we investigate whether CDC-seeded collagen scaffolds, cultured under hypoxia to induce release of angiogenic growth factors, can improve cardiac function when applied to the infarcted rat heart.

Methods Porous collagen scaffolds were prepared by freeze-drying a suspension of type 1 collagen (1% wt/v in 0.05M acetic acid (pH 3.2)) on a smooth film prepared by air-drying the collagen suspension. CDCs were isolated and expanded from male Sprague Dawley rats according to published protocols (Carr *et al*, PLoS One 2011). 10^5 CDCs were seeded onto the porous collagen scaffolds and cultured in hypoxia (5% oxygen) for 7 days. Myocardial infarction was induced in female Sprague Dawley rats with reperfusion after 40 minutes, at which point the scaffold was sutured to the infarct region of the heart. Hearts (4 groups: sham, infarct, infarct+scaffold, infarct+scaffold+cells, $n = 5-6$ per group) were scanned *in vivo* using MRI at 1 week, 6 weeks and 12 weeks post MI.

Results CDC-loaded scaffolds cultured under hypoxia released significantly more VEGF than those cultured under normoxia. Bi-layer scaffolds could be sutured to the myocardium with the collagen film on the out-facing surface to reduce adhesion to the chest wall. Scaffolds without cells were attached to the infarcted heart for 2 or 6 weeks. At 2 weeks the scaffold had not fully integrated with the myocardium but by 6 weeks scaffolds were integrated and had begun to be degraded. Histological analysis showed substantial macrophage infiltration at 2 weeks and formation of blood vessels within the scaffold after 2 and 6 weeks. MRI of all four treatment groups showed that ejection fraction was significantly lower in all infarcted hearts at all time points but there was no significant difference in cardiac function between untreated infarcted hearts and those treated with scaffolds, with or without cells. By 12 weeks, in the majority of cases, no remnants of the scaffold could be detected by visual inspection.

Conclusion CDC-loaded collagen scaffolds could be attached to the infarcted heart but did not improve cardiac function. Scaffolds took time to merge with the myocardium although infiltration of macrophages and blood vessels occurred before scaffolds were fully integrated.

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IN SITU EXAMINATION OF PLAQUE MACROPHAGE POPULATIONS USING MULTICOLOUR FLORESCENCE MICROSCOPY REVEALS CRITICAL DIFFERENCES BETWEEN MURINE MODELS OF EXPERIMENTAL ATHEROSCLEROSIS

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Macrophages play a central role in the development of atherosclerosis. They are highly plastic and heterogeneous cells that exhibit a spectrum of phenotypes, from pro-inflammatory or 'classically activated' to anti-inflammatory or 'alternatively activated'. Plaque macrophages in particular have roles in the pathogenesis of lesion stability and rupture, the most common cause of cardiac associated mortality. Despite considerable work aiming to characterise these phagocytic cells in disease,

our knowledge of their complexity *in vivo* is only partial. Here we wished to characterise plaque macrophages from two of the most common murine models; ApoE^{-/-} (Apolipoprotein E null) and LDLR^{-/-} (low density lipoprotein receptor null) and a relatively new and less characterised model; PCSK9-AAV8 (Adeno-associated virus serotype 8-proprotein convertase subtilisin kexin type 9) induced hyperlipidaemia.

Using immunohistochemistry, plaques from high-fat diet fed ApoE^{-/-}, LDLR^{-/-} and PCSK9-AAV8 mice were simultaneously stained for the pan macrophage marker Mac-3 and the pro-/anti-inflammatory markers inducible nitric oxide synthase (iNOS) and Arginase I (ArgI). Plaques were imaged using fluorescence microscopy and analysed by ImageJ. Individual Mac-3⁺ cells were selected as the region of interest and corresponding iNOS and ArgI staining was quantified. The analysis allowed consideration for the spectrum of marker co-expression and characterisation of individual cells based on staining intensity. Using this approach, complex populations of plaque macrophages including single+ ArgI, double positive, double negative and single+ iNOS were quantified. To understand the roles of these populations in atherosclerosis further, we correlated macrophage quality and quantities with lesion size and collagen content.

We show that ApoE^{-/-} plaque macrophages are significantly more pro-inflammatory than LDLR^{-/-} and PCSK9 plaque macrophages ($p < 0.05$ and $p < 0.0001$). The population responsible for the pro-inflammatory phenotype of ApoE^{-/-} macrophages were single+ iNOS cells ($p < 0.0001$). We also show that the abundance of these cells significantly correlates ($R^2=0.4791$, $p = 0.0183$) with lesion size in the aortic sinus. In addition, the frequency of double negative macrophages correlated with lesion collagen content ($R^2=0.4451$, $p = 0.0178$).

For the first time, plaque macrophages from three murine atherosclerosis models have been comprehensively characterised using a multi-colour image analysis strategy and suggest that plaque macrophages from ApoE^{-/-} mice are significantly more pro-inflammatory than LDLR^{-/-} and PCSK9 macrophages. We show that single iNOS⁺ cells may have a role in promoting lesion formation and double negative cells may also have a role in lesion stability. We envisage our platform provides a novel tool to gain a further, in-depth understanding of macrophage phenotype in atherosclerosis and will use it to elucidate the action of modulators of macrophage polarisation *in vivo*.

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HDAC3 UNCONVENTIONAL SPLICING MEDIATES ENDOTHELIAL-MESENCHYMAL TRANSITION IN CARDIAC FIBROSIS

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Introduction Endothelial-mesenchymal transition (EndMT) is a process where endothelial cells (ECs) undergo dramatic cytoskeletal remodelling and gene expression changes, leading to a more motile mesenchymal phenotype that can significantly contribute to cardiac fibrosis. Histone deacetylase 3 (HDAC3), a class I HDAC, is essential in the maintenance of endothelial homeostasis. Our previous study indicates that mouse HDAC3 mRNA can undergo unconventional splicing, and that the HDAC3-alpha (HDAC3a) spliced isoform promotes EndMT in

human aortic endothelial cells (HAECs). This project aims to elucidate the role of HDAC3a in mediating EndMT and its underlying mechanisms in the development of pressure-overload-induced cardiac fibrosis.

Methods and results Our previous study reveals that HDAC3a promotes EndMT in HAECs via activation of transforming growth factor-beta 2, which could possibly be mediated through increased protease enzyme activity. Therefore HAECs overexpressed with HDAC3a via adenoviral gene transfer (Ad-HDAC3a) have undergone a protease array, which revealed that cells overexpressed with HDAC3a have an elevated level of a disintegrin and metalloproteinase with thrombospondin motifs 1 (ADAMTS1). This was further confirmed with increased mRNA and protein expression levels of ADAMTS1 in Ad-HDAC3a-infected HAECs using RT-qPCR and Western blotting analysis respectively (both $n = 4$, $P < 0.05$). A mouse model of pressure overload-induced cardiac fibrosis was established by transverse aortic constriction (TAC), and cardiac fibrosis was confirmed by increased Col1a1 expression seen using RT-qPCR and Picrosirius red staining in heart tissue sections from mice 7 d post-TAC. Immunofluorescent staining detected CD31⁺/alpha-SMA⁺ (indicative of EndMT) and HDAC3a⁺/CD31⁺ cells in heart tissues from TAC mice but not in those from sham-operated mice. There were significant increases in mRNA expression of HDAC3a and ADAMTS1, together with EndMT transcription factors Snai1, Snai2 and Twist1 in heart tissues from 7 d post-TAC mice compared to those from 7 d post-sham mice ($n = 7-8$, $P < 0.05$). Elevated HDAC3a and ADAMTS1 protein expression were also detected in the TAC heart tissues ($n = 5$, $P < 0.05$).

Conclusion These results indicate an association between the HDAC3 splicing isoform HDAC3a, ADAMTS1 and EndMT during TAC-induced cardiac fibrosis. Further investigation of the underlying mechanisms mediated by HDAC3a and ADAMTS1 in cardiac fibrosis is warranted.

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THE MECHANISM OF P22PHOX C242T SNP INHIBITION OF TNF ALPHA-INDUCED NOX2 ACTIVATION IN HUMAN ENDOTHELIAL CELLS AND VESSELS

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NADPH oxidase, by generating reactive oxygen species, is involved in the pathophysiology of many cardiovascular diseases and represents a therapeutic target for the development of novel drugs. A single-nucleotide polymorphism (SNP) C242T of the p22^{phox} subunit of NADPH oxidase has been reported to be negatively associated with coronary heart disease (CHD) and may predict disease prevalence. However, the underlying mechanisms remain unknown.

Using computer molecular modelling we discovered that C242T SNP causes significant structural changes in the extracellular loop of p22^{phox} and reduces its interaction stability with the catalytic Nox2 subunit. Gene transfection of human pulmonary microvascular endothelial cells showed that C242T p22^{phox} reduced significantly Nox2 expression, but had no significant effect on basal endothelial superoxide (O₂⁻) production, or the expression of Nox1 and Nox4. When cells were stimulated with TNFalpha (or high glucose), C242T p22^{phox} inhibited significantly TNFalpha-induced Nox2 maturation,