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 monocytoysis *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,