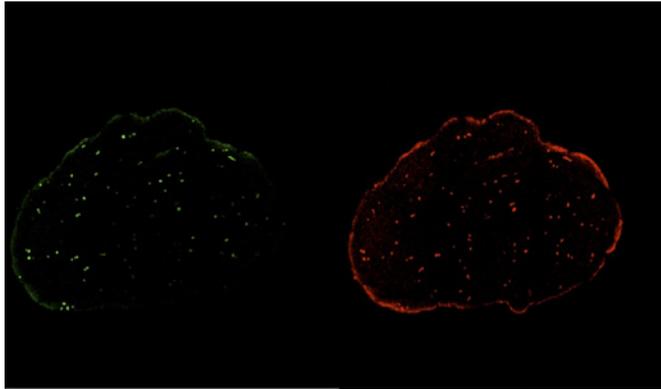
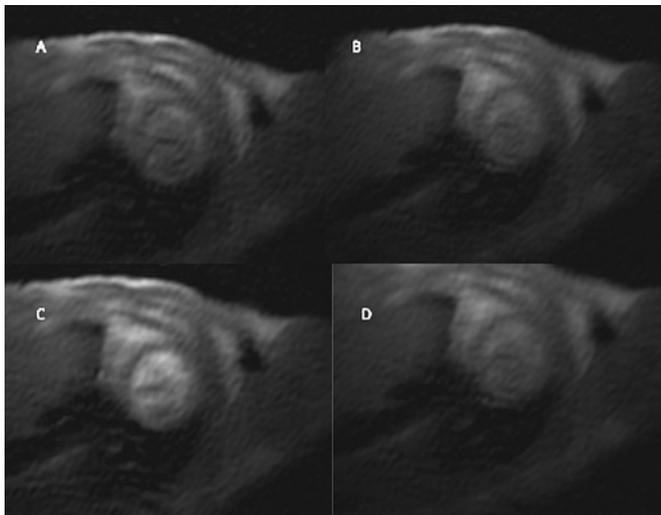


protocols, thus facilitating translation of imaging findings between rodent and human studies to elucidate mechanisms and develop therapies for cardiovascular disease.



Abstract 118 Figure 1 Fluorescent microsphere injections at rest (green) and stress (red).



Abstract 118 Figure 2 Murine stress perfusion (A)=rest (B)=RV first pass (C)=LV first pass (D)=myocardial wash in.

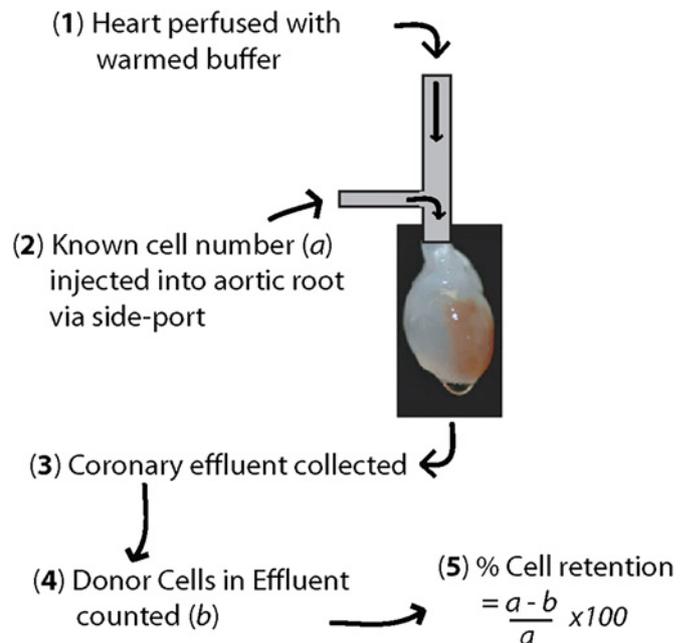
119 SIZE-DEPENDENT RETENTION OF STEM CELLS FOLLOWING INTRACORONARY INJECTION

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Background Intracoronary (IC) injection of bone marrow mononuclear cells (BMMNCs) is a promising treatment for heart failure. However, therapeutic effects in clinical studies have been small and a principle reason may be related to poor donor cell engraftment. Very early donor cell retention may be an important factor to determine engraftment, but this process is largely unexplored. This study aimed to quantitatively characterise early donor cell retention after IC injection using an original experimental model.

Methods A modified ex-vivo Langendorff perfused rat heart model was developed (Abstract 119 figure 1). Known numbers of rat BMMNCs were injected into the aortic root of the perfused heart ("normal hearts"). The same cell numbers were injected into hearts subjected to 30 min global ischaemia and 30 min reperfusion ("I-R hearts"). 8×10^6 rat BM-derived mesenchymal stem cells (MSC), a



Abstract 119 Figure 1

larger cell type, were also injected into normal hearts. Coronary effluent was continuously collected and cell numbers in the effluent were counted. In this way, the number of cells retained could be quantitatively calculated. The cell size distribution and expression of relevant cell surface markers, both for BMMNC pre-injection and for BMMNC in the coronary effluent, were respectively measured with an automated cell counter and flow cytometry.

Results Median size was 7.0 and 11.0 μm for BMMNC and MSC respectively. Most leakage of donor cells into the coronary effluent occurred within 2 min. Calculated retention ratios for the three BMMNC numbers were similar; approximately 20% in normal hearts increasing to 30% after I-R (Abstract 119 table 1). The retention ratio for MSC injection was much higher. Surface markers of BMMNCs were unchanged between pre-injection and coronary effluent cells. Instead, it was found larger BMMNC were more frequently retained in normal hearts (Abstract 119 table 2). This retention pattern was magnified in I-R hearts: larger BMMNC were retained 6 times more efficiently than smaller cells. Histological studies using PKH-stained BMMNCs demonstrated that all BMMNC were observed within the lumen of small vessels and none

Abstract 119 Table 1 Cell retention up to 5 min

Cell number injected	1×10^6	8×10^6	40×10^6
BMMNC—Normal Heart (%)	20.0 \pm 3.5	21.1 \pm 2.2	23.5 \pm 1.9
BMMNC—I-R Heart (%)	31.7 \pm 2.8*	30.9 \pm 1.2*	29.4 \pm 1.7*
MSC—Normal Heart (%)		77.8 \pm 4.1*	

Mean \pm SEM, n \geq 4 each group.

*p<0.05 vs same cell number BMMNC-Normal Heart.

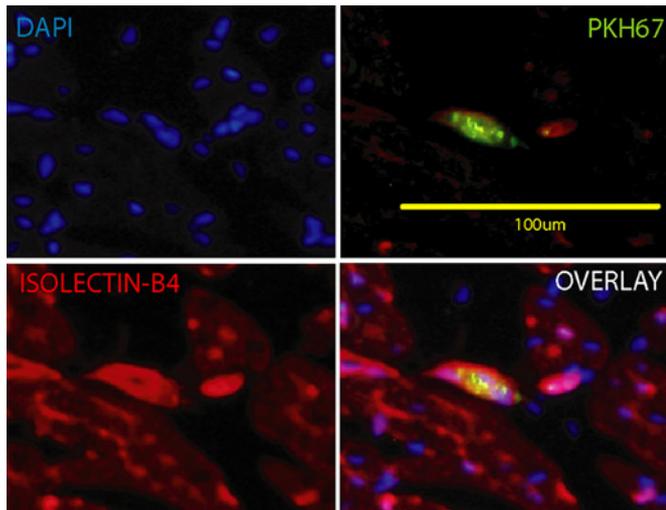
Abstract 119 Table 2 BMMNC-size dependent retention

Cell size	5–6 μm	7 μm	$\geq 8 \mu\text{m}$
Initial BMMNC fraction (%)	42.0 \pm 3.6	28.2 \pm 0.8	29.8 \pm 3.0
Retention up to 5 min (%)			
Normal Heart	14.5 \pm 7.3	30.3 \pm 4.2	39.8 \pm 6.1*
I-R Heart	9.5 \pm 2.6	40.5 \pm 2.8*	58.5 \pm 2.4**

Mean \pm SEM, n \geq 5 each group.

*p<0.05 vs 5–6 μm , **p<0.05 vs 7 μm .

were observed to have extravasated after 60 min (Abstract 119 figure 2). Coronary flow was not affected by injection of BMMNCs, whereas MSC injection led to a transient decrease of coronary flow.



Abstract 119 Figure 2

Conclusion Our study using an original model showed retention rates of BMMNC in normal hearts were low regardless of the cell number injected, suggesting a critical limiting factor for the treatment's success. Retention rates were improved by I-R. Larger BMMNCs were retained with an increased efficiency. This information provides intriguing potential methods for increasing retention in future clinical studies, thereby enhancing the therapeutic effect.

120 BLOOD FLOW IS REQUIRED FOR ENDOTHELIAL REPAIR IN A NOVEL IN VIVO MODEL USING ZEBRAFISH EMBRYOS

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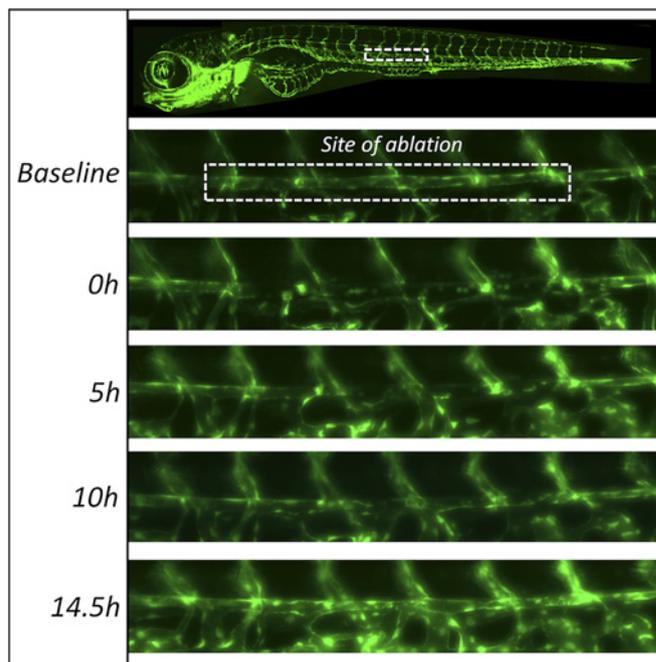
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Introduction Endothelial repair is essential for vascular homeostasis but difficult to study in vivo using existing animal models. The role of blood flow in the repair response remains unclear. We therefore established a novel in vivo model of endothelial repair using transgenic zebrafish embryos, and tested whether this was affected by the absence of blood flow.

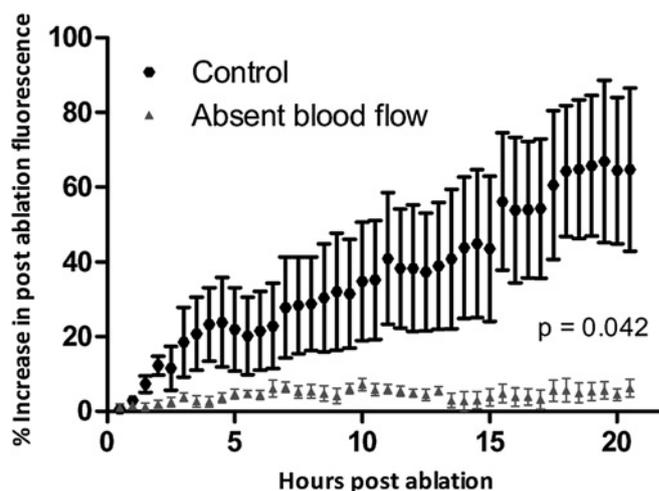
Methods 4-day old Fli1:eGFP transgenic embryos (expressing green fluorescent protein in endothelium) were anaesthetised and mounted in low melting point agarose. We used focused laser injury (Micropoint laser mounted on a Nikon inverted microscope) to ablate the endothelium of the dorsal aorta in the region between somites 15–17. Time-lapse fluorescence or confocal microscopy was then used to visualise recovery of the ablated endothelium in real time. To determine the role of blood flow in endothelial repair, embryos were treated with the anaesthetic tricaine which reversibly halts cardiac contraction and recovery from aortic ablation determined as above. This does not affect the embryos' development, which survive due to oxygen diffusion.

Results Abstract 120 figure 1 demonstrates endothelial ablation at 0 h. Over the following 14.5 h we observed migration of endothelial cells from both proximal and distal vasculature (aorta, vein, and intersegmental vessels) to repopulate the ablated region, suggesting this is a model of endothelial migration and proliferation. We did not observe recruitment of circulating cells to the site of injury. Complete recovery in all ablated embryos was achieved by 15–20 h post injury. Reversible cessation of blood flow significantly prevented endothelial repair compared with control (fluorescence at

20 h post injury: control $60\% \pm 22\%$ of baseline vs absent blood flow $5\% \pm 2.4\%$, $p < 0.05$ (Abstract 120 figure 2).



Abstract 120 Figure 1 Timecourse of endothelial repair following laser injury.



Abstract 120 Figure 2 Timecourse of endothelial repair in embryos with (black) and without (grey) blood flow.

Conclusions The ability to visualise endothelial damage and recovery in living zebrafish embryos offers a powerful new model for dissecting the mechanisms of endothelial repair in vivo. Endothelial repair requires blood flow even in the absence of recruitment of circulating cells.

121 BIOINFORMATIC IMAGING AND MOLECULAR INVESTIGATION FOR A ROLE OF P22^{PHOX} C242T POLYMORPHISM IN INHIBITING ENDOTHELIAL ROS PRODUCTION

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The p22^{phox} is a key component of the cytochrome b558 of the NADPH oxidase (Nox), which by generating reactive oxygen species