European Heart Journal (2017) **38**, 184–186
doi:10.1093/eurheartj/ehw056

EHJ BRIEF COMMUNICATION
Interventional cardiology

Intramyocardial stem cell injection: go(ne) with the flow

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Received 13 October 2015; revised 11 December 2015; accepted 25 January 2016; online publish-ahead-of-print 24 February 2016

In this study, we visualize the real-time dynamics of intramyocardial stem-cell injections. This shows a massive, immediate wash-out via venous drainage, accounting for the low retention. The use of carriers reduces this outflow.

Keywords Stem cell therapy • Intracardiac injections • Venous drainage

Many pre-clinical and clinical trials aim to deliver stem or progenitor cells in the heart, to protect or alleviate patients from the symptoms of myocardial infarction or heart failure. Although some beneficial effects on the heart have been reported,[1] the magnitude of effect is moderate and cellular retention is consistently low. Therefore, improvements in delivery strategies are needed in order to truly assess the regenerative potential of different injected cell types. Intracoronary and intramyocardial injections have been compared extensively, with no major differences detected in stem cell retention.[2] Interestingly, the majority of injected cells was found in the lungs rather than the heart, even after intramyocardial injection.[3] The mechanism underlying this observation remains unknown, as is the time window in which the cells are lost from the heart. The aim of this present study was to observe the events during and shortly after injection of stem cells in the myocardium.

For this, we injected two healthy, anaesthetized, female pigs with a mixture of 1 million mesenchymal stem cells (MSCs) and 0.2 million inert, cell-sized (15 μm) fluorescent microspheres using a 26G catheter placed in the centre of the cardiac muscle layers (*Figure 1A*).[2] To enable real-time visualization of fluid dynamics upon injection, the MSCs and microspheres were resuspended in contrast fluid (Telebrix) and fetal bovine serum (1:1 volume), without it affecting cell viability. After filling the injection catheter, total volumes of 500 or 200 μL cell suspensions were used for regular and concentrated injection volumes, respectively, thereby preventing an effect of the dead volume of the catheter. During and after injection, the localization of the contrast agent was recorded using C-arm fluoroscopy. In a third pig, we mounted 1 million MSC on large (60 micron) gelatin beads and injected these without contrast via the same method as described before. Five minutes after injection, all pigs were terminated by exsanguination.

Fluoroscopic monitoring of the contrast-resuspended cell suspension revealed the initial formation of a small depot at the tip of the injection catheter (Supplementary material online, *Movie S1*; red arrow in *Figure 1B*).[4] After 2–3 heartbeats, this depot was quickly emptied into nearby veins with each following contraction (Supplementary material online, *Movie S1*; green arrow in *Figure 1B*). This venous drainage is strongest during systole and decreases during diastole, when it is hardly visible using fluoroscopy. After 5 min, the depot was no longer visible (*Figure 1B*, lower-right panel).

Before, during and after the injection, blood samples were taken from the coronary sinus to detect the presence of the injected MSCs, which we had fluorescently labeled.[2] Upon blood sample collection, red blood cells were lysed and flow cytometric analysis was used for MSC detection. This demonstrated that wash-out of injected fluorescently labelled MSCs from the myocardium was most noticeable immediately upon injection (*Figure 1C* and *D*). This cell clearance was highest with the 500 μL injection (blue line), whereas the more concentrated sample (200 μL, red line) led to a lower immediate wash-out.

After collection of the heart, tissue samples were taken near the injection area, as well as from remote areas to search for the MSCs and the fluorescent inert microspheres, as shown in *Figure 1A*. Part of these samples were cryopreserved and used for microscopical

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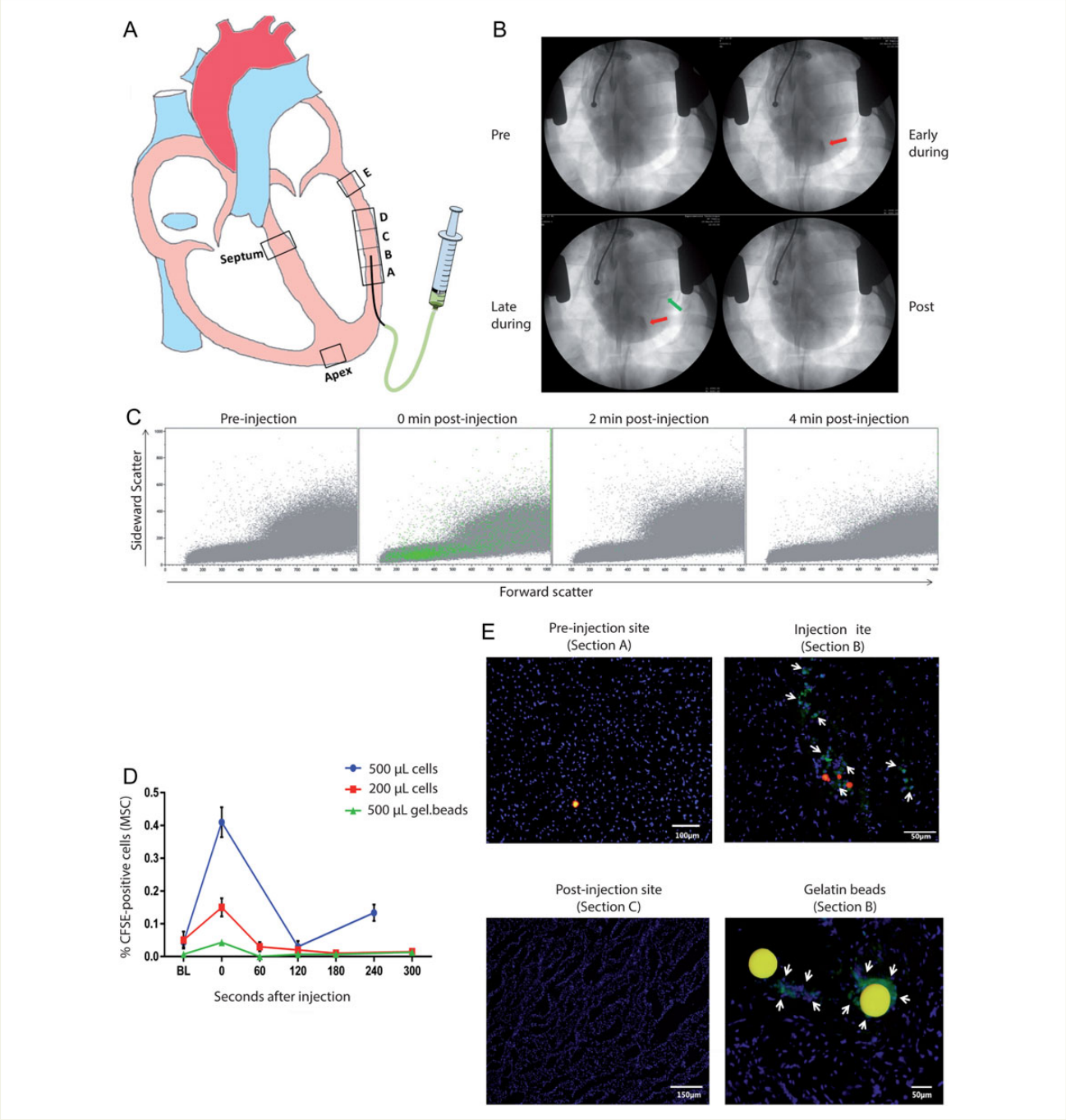

Figure 1 Stem cell retention in a porcine heart. (*A*) Experimental set-up: 1 million mesenchymal stem cells were labelled carboxyfluorescein succinimidyl ester (CFSE) and 0.2×10^6 red fluorescent beads were injection in the lateral wall of the porcine left ventricle (open thorax, Dutch Landrace pigs, 3 months of age, $\pm$70 kg). All animal experiments were performed in accordance with the Directive 2010/63/EU of the European Parliament, national guidelines on animal care and with prior approval by the Animal Experimentation Committee of Utrecht University). Labelled black squares indicate where tissue samples were taken for degradation and histology. (*B*) Contrast images depicting the localization of the injected fluid pre-, early during-, late during-, and post-injection. The red arrow points at the depot formed at the catheter tip, while the green arrow shows the venous outflow tract. (*C*) Forward-Sideward scatterplot of nucleated cells with fluorescently labelled mesenchymal stem cells marked in green. Coronary sinus samples were taken at different time-points before and after injection. (*D*) Quantification of the outflow of fluorescently labelled mesenchymal stem cells in the coronary sinus at different time-points after injection. The blood was exclusively sampled from the cardiac vein, as the hemi-azygos vein was ligated. $n = 1$ per group, error bars are standard deviations of technical replicates. (*E*) Histological analysis of pig heart samples. Images show the beads in the different sites of the heart. Colours indicate nuclei (blue; Hoechst), hemi-azygos (green; CFSE-label) and fluorescent microspheres (red) or gelatin beads (yellow). Both cells and fluorescent beads are visible near the injection site, present in the disrupted tissue. In more remote areas, no cells are retrieved. Bar = 100 µm.

detection of the labelled MSC, while the remainder were degraded for quantitative detection of the fluorescent microspheres.[5] The location of the co-injected inert fluorescent microspheres in the two hearts was determined by tissue degradation, where the majority of retrieved fluorescent beads was found near the injection site (data not shown). Small amounts were visible upstream of the catheter tip. This could be due to backflow along the catheter, or possibly an artefact due to catheter removal. The amount of fluorescent beads from the downstream areas from the injection site (areas C, D, and E) was comparable with the negative controls, apex, and septum, indicating limited diffusion of the fluorescent microspheres into distal myocardial tissue, including tissue along the 'outflow route'. In part of the tissue used for sectioning, MSCs were also found exclusively near the tip of the injection catheter, corresponding to the location of the depot previously imaged with contrast fluoroscopy. The local myocardial structure in this area was slightly disrupted and both the fluorescently labelled MSCs and the inert fluorescent microspheres were located in small clumps in these areas (*Figure 1E*, second pane). Like the microspheres, the MSC did not migrate to adjacent areas.

However, when MSCs were applied when attached *in vitro* to small gelatinous carriers (gelatin beads), the direct outflow into the coronary sinus was reduced 10-fold compared with its cells-only control (*Figure 1D*, blue vs. green line). Microscopical imaging of the injection site shows the increased presence of the MSCs, in close proximity to their gelatin carriers (*Figure 1E*).

Despite extensive pre-clinical and clinical trials, cardiac stem cell therapy displayed so far only modestly increased cardiac performance.[1] In the present observational study of the injection process, substantial numbers of MSCs were immediately flushed out of the heart via the venous system within a few heart beats after the start of injection. We realize that a limitation of this case report study is the small sample size of merely one animal per group. Furthermore, the contrast can only be optimally imaged with open chest and dilution by natural muscle perfusion might lower the signal in the course of time. Still, to our knowledge, this is the first report that demonstrates the dramatic wash-out of intramyocardially injected cells via these visualizations. This study clearly demonstrates the major limitation of current cell delivery approaches and thereby potentially also their observed limited functional benefits to the heart. Although positive effects on cardiac function have been reported, increasing the retention of the cells will likely improve these effects.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Authors' contributions

F.v.d.A. performed statistical analysis. L.v.L., P.D., and J.S. handled funding and supervision. F.v.d.A., D.F., P.v.d.H., and E.v.E. acquired the data. F.v.d.A., P.G., P.D., and J.S. conceived and designed the research. F.v.d.A., D.F., and J.S. drafted the manuscript. I.H., G.P., S.C., and P.D. made critical revision of the manuscript for key intellectual content.

Acknowledgements

This works forms part of the Project P1.04 SMARTCARE of the BioMedical Materials Institute, co-funded by the Dutch Ministry of Economic Affairs, Agriculture and Innovation. The financial contribution of the Dutch Heart Foundation is gratefully acknowledged. This work was further supported by a grant from the Alexandre Suerman program for MD/PhD students of the University Medical Center Utrecht, the Netherlands, the ZonMw-TAS program (#116002016), and the Netherlands CardioVascular Research Initiative (CVON): the Dutch Heart Foundation, Dutch Federation of University Medical Centers, the Netherlands Organization for Health Research and Development, and the Royal Netherlands Academy of Sciences.

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Conflict of interest: S.A.J.C. is a co-founder of the CART-Tech company (www.cart-tech.com).

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