Does Surgical Intervention for Myocyte Transfection Impact on Cardiac Function in Mice?

E. Dall'Armellina[1], C. A. Lygate[1], H. Barnes[1], R. Carnicer[1], S. Neubauer[1], M. Markl[2], B. A. Jung[2], and J. E. Schneider[1]

[1]Department of Cardiovascular Medicine, University of Oxford, Oxford, Oxon, United Kingdom, [2]University Hospital Freiburg, Freiburg, Germany

Introduction: Direct intra-myocardial injections are often used for viral gene transfer experiments in the mouse. However, myocyte transfection by this route is inhomogeneous, localized mainly around the sites of injection [1]. This makes it difficult to detect an effect of the delivered gene by measuring global cardiac function, and functional data is instead often obtained from isolated myocytes. Tissue phase mapping (TPM) allows for an assessment of regional myocardial function [2,3] and therefore could be a useful tool to detect small changes to regional function *in vivo* after direct injection of genetic material. However, it is first necessary to determine whether the surgical intervention required to inject the heart has itself an effect on regional cardiac function and on baseline TPM parameters. We have therefore used TPM in mice to track changes in regional function at baseline, 24 hours and 8 days after direct intra-myocardial injection of saline.

Methods: Four C57BL/6 mice were anaesthetized with isoflurane in 100% O_2 and buprenorphine analgesia, then intubated and ventilated at 250µl stroke volume and 150 strokes/min. A thoracotomy was performed within the 5th intercostal space, left lung collapsed, and the pericardium opened. A 5/0 suture was temporarily placed in the apex to allow manipulation of the heart. A total of 6x 10µl saline injections were given at regular intervals to the upper half of the left ventricular free wall. The lung was then re-inflated, wound closed, and animal allowed recovering.

Murine TPM experiments were performed on a 9.4T VNMRS DirectDrive MR-system (Varian Inc, USA), equipped with a quadrature driven birdcage coil (id 33mm). Data of three contiguous slices in short axis view were acquired using a double-gated black blood cine gradient echo sequence with three-directional velocity encoding (128 x 128, FOV 25.6 x 25.6 mm, α=10°, 2 averages, venc in-plane 6 cm/s; through-plane 8 cm/s). For evaluation of ejection fraction (EF) and stroke volume (SV), additional slices covering the remaining ventricle to the apex (3-4 slices) were acquired using the same sequence without velocity encoding.

Data post-processing using customized Matlab software, included contour segmentation, a correction for translational motion components and a transformation of in-plane velocities into polar coordinates. As a result, motion parameters are decribed in terms of radial, rotational and longitudinal velocities. The LV was divided into 24 angular areas for which radial velocities were averaged and correlated to a reference time course based on the global velocity time course averaged over the whole segmentation mask [4]. Positive correlation coefficients (*cc*) correspond to similar or hypokinetic motion patterns, while values near zero describe akinetic motion and negative values express dyskinetic waveforms.

Results: Fig. 1 shows velocity vector field plots (unprimed) and color-coded maps of radial velocities (primed) in a mid-ventricular slice for four characteristic time frames during the cardiac cycle of a mouse corresponding to (A, A') baseline, (B, B') 1 day and (C, C') 8 days post surgery, respectively. Fig. 2 depicts *cc* plots for the same slice shown in Fig 1. Prior to surgery, baseline cardiac function showed no regional heterogeneity. After surgery, only the anterior wall exhibited a relative impairment in regional function (arrow), which was observed in all four mice at 24 hours. This was resolved in 2 out of four mice at 8 days post-surgery. Importantly, no change in global cardiac function (i.e. EF (filled symbols) and SV (open symbols)) could be detected (Fig. 3 – mean ± SD, n=4).

Discussion: This work demonstrates the value of assessing regional cardiac function using TPM in mouse models. While EF and SV didn't show any detrimental change pre- compared to post-intervention, a relative impairment (change in motion pattern) of the anterior wall was found post surgery and injection. Lateral and posterior walls were also injected but did not develop localized dysfunction, suggesting this does not represent cellular damage at the sites of injection. However, more animals and extended investigations including regional time courses (peak velocities, time-to-peak) or longitudinal *cc* are needed to confirm this finding. Instead we hypothesize that impaired function in the anterior wall is related to removal of the pericardium, which allows direct contact between this region and the thoracotomy wound. Dysfunction could arise due to focal adhesion to lung or chest wall, or to release of inflammatory mediators from the incision site.

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Figure 1:

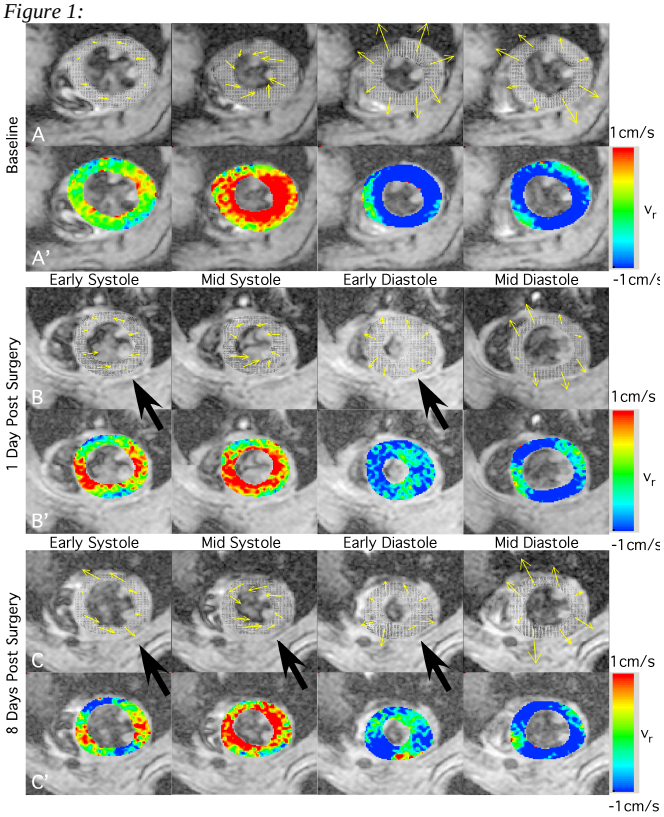


Figure 2:

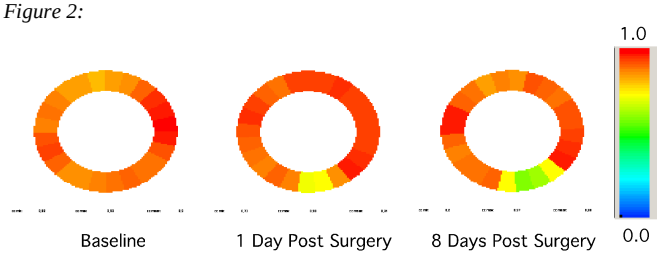
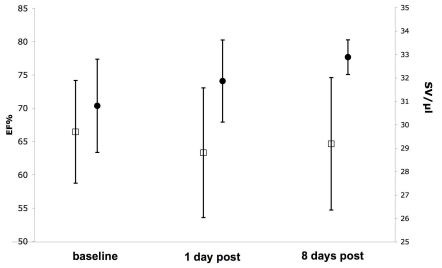


Figure 3:



References:

[1] Muller OJ et al. Cardiovasc Res 2007;73:453-462. [2] Herold et al. MRM 2006;1058-64.
[3] Jung et al. JMRI 2006;1033-39. [4] Markl et al. JMRI 2002;15:642-53.