µMRI Optimisation for Phenotyping the Embryo Mouse Heart

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Introduction: Transgenic mice are an integral part in the study of gene function in cardiac development and disease. Determining the phenotype of different transgenic lines is crucial. µMRI is an emerging technique for high-throughput screening of late-gestation mouse embryos [1]. Most methods of embryo imaging rely on fixation with gadolinium-chelate contrast agent in addition to fixative, resulting in a large reduction of tissue T_1[2]. This leads to higher signal when using short TR sequences, such as 3D gradient echo, for high-resolution scans. However, there is little data on appropriate fixation time, Gd concentration and contrast optimisation for gradient echo imaging of embryos in the literature. We present a study to improve contrast in the embryo heart through preparation and MR scan parameter optimisation. The results were then applied to an MRI screen of embryos heterozygous for the gene *Chd7*, known to be implicated in human CHARGE syndrome – a condition characterised by the presence of congenital heart defects [3].

Methods: *Embryo Preparation:* E15.5 CD-1 embryos were fixed in a solution of 4% PFA and Gd-DTPA. Left for either 3 days or 2 weeks in 2, 4mM and 2, 4, 8, 16mM Gd-DTPA respectively and embedded in 1% agarose doped with an identical Gd-DTPA concentration. 13 embryos (5 *Chd7*$^{+/+}$, 8 *Chd7*$^{+/-}$), also at E15.5, were prepared using a Gd-DTPA concentration of 8mM. Imaging was performed on a Varian 9.4T VNMRS System with a 39mm volume coil (RAPID Biomedical GmbH). *T_1 & T_2* Mapping:* *3-day:* 10 TIs (IR-SE, TE=17ms): 20 to 5000ms; 10 TEs (GE): 10 to 120ms; *2 week*: 10 TIs: 7.5 to 900ms; 10 TEs: 7 to 43ms. The resulting images were then fitted to generate T_1 and T_2* maps.
Heart Contrast Simulations: Using 2-week data, T_1 and T_2* were measured from ROIs in heart and background agarose (a surrogate for heart chamber free fluid). To determine the optimum parameters for maximum heart contrast (Contrast = $Signal_{heart}$–$Signal_{chamber}$) the gradient echo signal equation was incorporated into a Matlab (Mathworks Inc., Natick MA, USA) simulation. Using T_1 and T_2* at each concentration, together with a range of TEs, TRs and flip angles, the optimum imaging parameters were calculated, using maximum contrast estimation.
Volume Imaging and Image Processing: 3D gradient-echo scans were acquired (27x27x27mm FOV, 512^3 matrix) for each concentration to review optimum MR parameters found by the simulation.

Results: Compared to 3-days, 2-week fixation showed reduced mean T_1 in the heart at both 2 and 4mM concentrations ($p<0.0001$, maps not shown), resulting in calculated signal gains of 23% (2mM) and 29% (4mM). Fig. 1 shows SNR measurements in tissue volumes from 2-week data acquired according to optimum simulated MR parameters. We confirmed experimentally that while 16mM produced the best contrast, fixation with 8mM Gd-DTPA (TE/TR/FA/NSA=9/20/60/7) produced the greatest heart SNR (23.2±0.96) and good heart-chamber contrast (CNR 27.1 ±1.6) without the major susceptibility artifacts seen at 16mM. Fig. 2 shows a volume rendering of the heart and major vessels based on 8mM data. In an embryo screen we identified a ventricular septal defect in an embryo heterozygous for *Chd7* (Fig. 3).

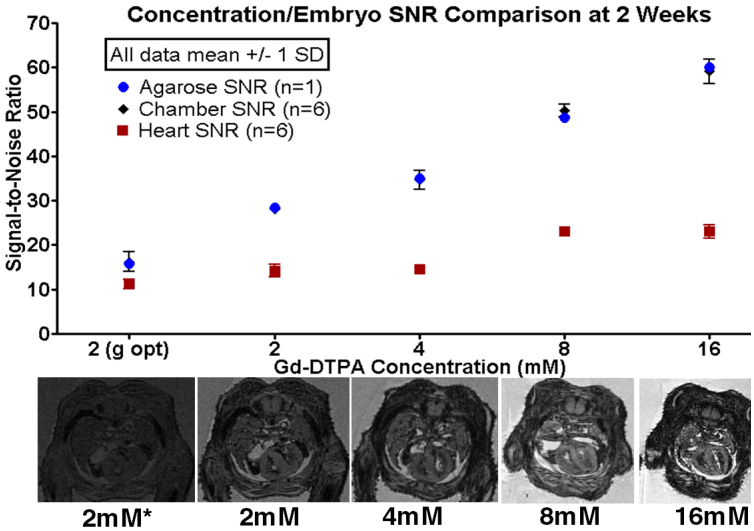

Fig 1: *Measured SNR in three volumes of interest in two-week fixed embryos at four Gd-DTPA concentrations. 2mM embryos were also imaged at global optimised parameters for the whole-embryo taken from the literature[4] (*). Images equally scaled.*

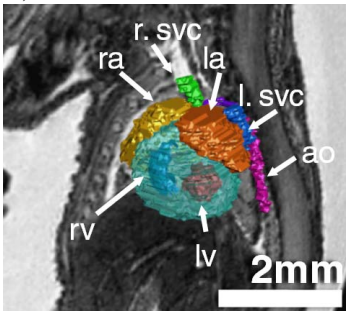

Fig 2: *Volume rendering of embryo heart at 8mM showing major structures: atria (la, ra), ventricles (lv, rv), aorta (ao) and superior vena cavae (l.svc, r.svc)*

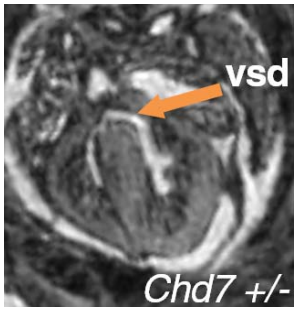

Fig 3: *Axial slice in an embryo heterozygous for Chd7, showing a ventricular septal defect (vsd).*

Conclusion: Increasing fixation time from 3 days to 2 weeks resulted in a significant reduction in T_1 and thus signal gain in 3D GE imaging. At two weeks fixation, increased Gd-DTPA concentration is an effective method of increasing SNR and CNR in the heart. Although 16mM would appear to offer the greatest signal difference between heart muscle and chamber (Fig. 1), susceptibility artifacts in the tissue were particularly prominent at this concentration due to the short T_2*. This study indicates that an 8mM concentration, in combination with 2 weeks fixation and the above suggested MR parameters, offers the best heart tissue SNR and heart-chamber contrast, without compromising image quality. Using these optimum parameters we visualised a heart defect in a screen of *Chd7*$^{+/-}$ embryos (Fig. 3). Our optimisation should allow greater sensitivity to such defects in future heart phenotyping studies.

Acknowledgements: Dr. Karen McCue and Sarah Beddow, UCL Institute of Child Health, for transgenic embryos. Dr. Jurgen Schneider and Prof. Shoumo Bhattacharya, University of Oxford for helpful discussions. Dr. Andrew Lowe for assistance with T_1 and T_2* mapping. Our work is supported by the BHF, BBSRC and EPSRC.

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