Heart transplant recipients require frequent invasive endomyocardial biopsies, which carry a significant risk of complication and are prone to sampling errors. Although newer immunosuppressive therapies continue to improve survival rates, organ rejection still occurs and close surveillance of the graft is mandatory. There is, thus, a compelling need to develop (1) better immunosuppressive and tolerogenic strategies and (2) noninvasive, more predictive, and quantitative diagnostic tools to monitor individual patients and to test new treatment regimens in animal models and patient cohorts. Targeted molecular imaging approaches offer the potential for more sensitive early detection of transplant rejection.1–4 Such emerging strategies, however, have to be validated in murine models that resemble rejection of the working human heart.

The retrogradely perfused, beating but nonworking heterotopic heart transplant model in the mouse has been extensively used in more than 30 years of research to address basic immunologic questions of rejection and tolerance because the mouse is the preferred species for basic immunology research. To our knowledge, to date, there have been no reports on working heart mouse models. The nonworking murine model has several physiologic limitations, because there is no forward flow through the grafted heart resulting from anastomosis of the donor aorta to the recipient aorta and the donor superior vena cava to the recipient inferior vena cava (IVC). Imaging studies as well as research into new immunosuppressive strategies would greatly benefit from a more realistic murine model with blood flow and cardiac workload conditions closer to human orthotopic transplantation. Orthotopic transplantation is technically challenging in the mouse, mainly because miniaturized bypass machinery is currently unavailable. We have, therefore, developed a new surgical technique of heterotopic combined heart/lung transplantation that results in a working heart, which can be imaged to assess functional parameters, such as ejection fraction and wall motion (Figure).

The institutional subcommittee on research animal care approved all animal studies. During deep isoflurane anesthesia, we explanted donor hearts together with the right lung after ligation of the IVC and the left pulmonary arteries and veins from C57B/6 mice (Figure). In the recipient C57B/6, a median abdominal incision exposed the abdominal aorta and IVC, which were side clamped. We then performed an end-to-side anastomosis of donor aorta to recipient aorta and an end-to-side anastomosis of the donor superior vena cava to the recipient IVC (Figure D and Movie 1). After unclamping of the recipient aorta and IVC, the donor heart spontaneously resumed beating at a heart rate slightly lower than the orthotopic heart (Movie 2). We have now reached a surgical success rate of ≈90% (n=8). This surgical approach is similar to the one described by Ho and coworkers, who performed block heart and lung transplants in rats.1–3 These surgeries are technically difficult even in the rat (10× the body weight of the mouse), and it was unknown whether they could be adapted to murine physiology. Furthermore, in their earlier work, the superior vena cava was not anastomosed6 as in the later publications6 or in the technique described here.

Compared with the nonworking model (Movie 3), the original direction of blood flow through the heart and the lung is preserved (Figure A), and the heart is pumping blood. This has 2 major advantages: (1) the overall situation of the heterotopic graft better approximates orthotopic transplantation, and therefore, data are more translatable, and (2) because the heart is working, one can measure regional and global function with tagged and cine MRI (Movies 4 and 5) and, in the future, investigate if targeted molecular imaging approaches can detect rejection before function declines. The movies also highlight the potential of noninvasive quantitative imaging of basic transplant rejection studies. Traditionally, the only noninvasive read out is abdominal palpation of the recipient mouse, and the quality and frequency of the heart motion are described using a 4-level beating score. This index may be observer dependent and has a limited dynamic range (score 1 to 4), whereas imaging can detect subtle changes in ejection fraction or even regional myocardial dysfunction in tagging MRI observer independently.

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Disclosures
None.

References

Figure. A, the surgical approach and direction of blood flow (arrows) are shown. B, The explanted donor heart/lung package is stored in ice-cold cardioplegic solution before implantation into the recipient. C, Surgical field after transplantation (magnification ×2). D, Vascular anastomoses (magnification ×4). E, Typical ECG trace recorded during imaging. The gradient noise is visible during every other heart cycle. The ECG of the heterotopic graft can be isolated from the orthotopic recipient heart by attaching leads to the hind legs of the mouse. F and G, Diastolic and systolic mid-ventricular cine MRI frames. MRI was performed on day 1 after isografting using isoflurane anesthesia (1.5%, O₂ 2 L/min) on a 7-T scanner (Bruker Pharmascan, Billerica, Mass). Cine images were obtained with ECG and respiratory gating (SA Instruments, Stony Brook, NY) using a gradient echo cine sequence and a dedicated mouse cardiac birdcage coil (Rapid Biomedical, Wuerzburg, Germany) with the following imaging parameters: echo time, 2.7 ms; 16 frames per RR interval (repetition time, 10 ms); in-plane resolution, 200×200 μm; slice thickness, 1 mm; number of excitations (NEX), 4. H and I, Diastolic and systolic mid-ventricular frames from a tagged cine MRI acquisition. Tagging was achieved with a spatial modulation of magnetization (SPAMM) prepulse of 17.8-ms duration immediately after the QRS trigger, with 1 mm distance between taglines and 0.2 mm line thickness. All other acquisition parameters were identical to those described earlier for the nontagged gradient echo cine. PA indicates pulmonary artery; PV, pulmonary vein; LV, left ventricle; RV, right ventricle.


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**SUPPLEMENTAL MATERIAL**

**Movie captions**

Movie 1: High magnification movie (4x) of vascular anastomoses. Movies are best watched with quick time movie player.

Movie 2: The graft spontaneously resumes beating after unclamping restores blood flow (magnification 2x).

Movie 3: Mid-ventricular cine MRI of traditional non-working model shows very reduced myocardial motion. In this model, the left ventricle is typically filled by a blood clot. No bright blood signal is observed inside the LV cavity, because there is no spin refreshment due to absent flow.

Movie 4: Mid-ventricular tagged cine MRI of working heart graft shows the feasibility of quantifying regional myocardial contractility.

Movie 5: Mid-ventricular cine MRI of working heart graft shows feasibility of measuring global LV function.