Visualization and quantification of whole rat heart laminar structure using high-spatial resolution contrast-enhanced MRI


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Myocardial structure is centrally important to cardiac mechanical function in health and disease (6), and myolaminar sliding is thought to be the principle mechanism of myocardial thickening in systole (10). Myolaminar structure has recently been shown to substantially influence the spread of activation in the myocardium (8, 20). The myocardium is structured as stacked laminae of myocytes 4–6 cells thick (~80–120 μm), also known as myolaminae or sheets (24). These laminae are organized together in a complex fashion in which there are some regions of abrupt transmural change in laminar organization. The long axes of the myocytes, which make up the laminae, have a regular helical organization: the orientation of their long axes (with respect to the cardiac short axis) varies through ~120° transmurally from endocardium to epicardium (33). The average orientation of the long axes of neighboring myocytes is known as the fiber-orientation (16). The myocardium therefore has regular lower order fiber architecture, with a more irregular and locally distinct higher order laminar architecture. Due to the presence of myofiber and myolaminar structure, at each point in the myocardium three principal orthogonal structural directions can be defined: 1) along the fiber axis, 2) perpendicular to the fiber axis in the sheet plane, and 3) normal to the sheet plane. This is known as orthotropic structure (8). These orthogonal structural axes influence the electrical coupling and the spread of activation of the myocardium, which is in the approximate ratio 4 to 2 to 1 in the directions along fiber-axis to within the sheet plane to normal to sheet plane (8, 20).

Spotnitz et al. (32) recognized that myocardial thickening cannot be accounted for by changes in cardiomyocyte geometry alone. They studied the contribution made toward myocardial thickening by each of the myofiber diameter and the sliding between bundles of myocytes. They concluded that cleavage planes were present between groups of myocytes and that the sliding of groups of myofibers permitted by the cleavage planes was an important mechanism in myocardial contraction, and furthermore showed that populations of cleavage planes have a more vertical alignment in diastole and a more horizontal alignment in systole. This has become known as the Sheet Sliding Hypothesis, and it is supported by studies using cinematic radiography (10), force transducers (12), diffusion tensor-MRI (DT-MRI) (9), and computational simulation (3) (reviewed in detail in Ref. 16). Evidence from histology and DT-MRI (a method described below) has shown that sheets belong to two primary populations (of positive and negative orientation with respect to the cardiac short-axis), have a complex distribution throughout the cardiac wall, and have localized regions of sudden change in orientation (3, 9, 16, 18).

Myolaminar structure has been imaged using conventional histology (24); extended volume confocal microscopy (29, 35); and DT-MRI (16, 18), an MRI method where structure is measured through the restriction of free water diffusion (16, 21, 30, 34). Potentially myolaminar structure could also be...
imaged using confocal microscopy after myocardial optical clearing (31). Histological imaging methods are limited to postmortem application, destroy the tissue imaged, and cannot be developed toward in vivo application. Two-dimensional histological methods have limited potential to measure out-of-plane structure, and three-dimensional histological methods require time-consuming tissue preparation. The use of DT-MRI for measuring cardiac laminar architecture has been reported in a small number of studies (30, 34); however, validation of the approach has been performed by comparison with a small number of histological measurements. It is our view that DT-MRI measurement of laminar architecture requires further thorough quantitative validation. Diffusion imaging methods measure laminar orientation indirectly, producing an abstract map of anisotropy/orthotropy, and as such the image data are fundamentally different from a direct three-dimensional imaging approach. A significant advance in imaging myolaminar structure came with the development of high-resolution T2-contrast MRI (22, 23), where it was demonstrated that high-quality images of cross sections of myolaminar architecture could be acquired during the diastolic phase of beating isolated perfused rat hearts, and good qualitative correspondence to histology was demonstrated. Extension of high-resolution direct (nondiffusion) MRI to the whole ventricular volume and to the study of the three-dimensional architecture of myocardial laminar architecture has not yet been performed, and this MRI approach has not been quantitatively validated against histology.

We hypothesized that MRI of myocardial laminar architecture, as has thus far been applied to thin slices of myocardium, could be applied to whole fixed hearts. Furthermore we hypothesized that perfusing hearts with contrast agent (Gd-DTPA) would provide enhanced contrast between myolamines and cleavage planes and higher signal-to-noise ratio (SNR), allowing high-quality high spatial resolution images of the whole-ventricular myolaminar structure to be acquired. After imaging we used conventional histology to quantitatively validate the structures observed and measured in our MR images. We then compared the structure between rat hearts to quantify interindividual variability. The aim of this study was to develop an MRI method of quantifying whole-ventricular laminar structure. In principal, this approach could be used to quantitatively understand cardiac physiology and pathophysiology in animal models, and perhaps could be adapted to implement clinical personalized heart models that will be used for the planning of clinical electrophysiological interventions.

MATERIALS AND METHODS

Heart preparation and perfusion fixation. Male Wistar rats (n = 4) weighing 200–220 g were euthanized in accordance with the UK Home Office Animals (Scientific Procedures) Act 1986 and the U.S. Government Principles for the Utilization and Care of Vertebrate Animals, with the approval of the UK Home Office and the Local Ethics Committee. Hearts were rapidly dissected and the aorta cannulated, and the hearts were perfused retrogradely at 7 ml/min with HEPES-Tyrode solution maintained at 37°C for 5 min on a Langendorff apparatus to allow beating to resume and to assist clearing of blood. The HEPES-Tyrode solution consisted of (in mmol/l): 130 NaCl, 5.4 KCl, 0.4 NaH2PO4, 1.4 MgCl2·6H2O, 5 HEPES, 10 glucose, 20 taurine, 10 creatine (pH adjusted to 7.4 with NaOH), and 0.75 mM CaCl2 and containing 0.1% Heparin. Cardiac contraction was then prevented by perfusion with Tyrode solution containing 0 mM CaCl2 and 10 mM 2,3-butanedione monooxime for 5 min. Next, the hearts were simultaneously perfused with MRI contrast agent and fixative for 20 min, using Tyrode solution containing 0 mM CaCl2, 4% formaldehyde, and 0.1% Gd-DTPA (dimeglumine gadopentetate Magnevist, Bayer Schering Pharma). The hearts were then removed from the perfusion apparatus and stored for between 4 and 12 h in the contrast/fixative solution until imaging.

MRI acquisition and reconstruction. All hearts were imaged using a 1.5T T1 weighted FLASH (Fast Low Angle SHot) MRI sequence (15) in a Bruker (Ettlingen, Germany) 9.4T spectroscope with 20 averages and echo time (TE) = 7.9 ms, repetition time (TR) = 50 ms, and flip angle (α) = 40°, taking a total of 18 h to acquire at a resolution of 50 × 50 × 50 μm, a matrix size of 256 × 256 × 512 for a field of view of 12.8 × 12.8 × 25.6 mm. One heart (C1) was then imaged using the same imaging sequence with TE/TR = 13.2/50 ms, 20 averages taking a total of 72 h to acquire at a resolution of 25 × 25 × 37 μm, a matrix size of 512 × 512 × 512 for a field of view of 12.8 × 12.8 × 19.2 mm. This method produces similar contrast with that described previously (23), except that to enhance SNR, Gd-DTPA T1-contrast is mapped rather than absolute native tissue T1-values.

Histology. Within 12 h of MRI rat heart C1 was frozen in isopentane (2-methylbutane; Sigma), stored at −80°C, mounted using optimum cutting temperature (OCT) compound (VWR) and cryosectioned. Long-axis coronal sections were cut at 16 μm thickness and were mounted on Superfrost Plus glass slides (VWR). Frozen sections were fixed in Bouin’s fluid (Sigma) and washed in 70% ethanol and then stained with Masson’s trichrome as described previously (11).

Fig. 1. A: light microscope image (digital microscope, Dino-Lite AM-2011; AnMo, Taiwan) of a transmural cut through an unloaded ventricular wall. B: schematic representation of cardiac location of the image in A. A movie from which the still image in A was obtained is available in Supplemental Material. In this movie the slide of the myolaminar structure accompanying myocardial contraction is seen and demonstrates that laminar structure is readily observed in the unloaded ventricular wall. RV, right ventricle.
After staining, tissue sections were dehydrated through graded ethanols (70% to 100%), cleared in Histoclear, and permanently mounted in DPX mounting medium (BDH). Sections were stored at 20°C, and subsequently a light microscope with attached digital camera (Carl Zeiss, Imager Z1) was used to acquire the images and AxioVision software (Carl Zeiss) was used to collect and stitch the images. Orientation analysis was carried out as described below for the MR images.

MRI segmentation, registration, and quantitative digital image analysis. The images were segmented and the background removed using Seg3D (Scientific Computing and Imaging Institute, University of Utah) as described (5). The ventricles were manually segmented from the atria using Seg3D. All datasets were registered to a chosen reference dataset (denoted as C1) using Slicer3 (26). Affine registration was used to generate one series of images (aC2, aC3, aC4), and deformable registration (B-spline registration) was used to produce a second image series (dC2, dC3, dC4). Histology images were deformably registered to the MRI (28), as described in detail below. In-plane sheet orientation was measured using Quantitative Orientation Analysis (14), which is based on evaluation of the Structure Tensor of local image neighborhood contrast and is implemented as an analysis tool through the ImageJ environment (1). The method provides a quantitative measure of orientation and anisotropy. Coherence (C) is a measure of the degree of anisotropy; when C = 0 the image is isotropic and not orientated, and when C = 1 there is a single dominant orientation. Regions where the structure tensor coherence C < 0.1 have been excluded since there is not high confidence in the measured orientation. It is common to report sheet-orientation with respect to cardiac specific frames of reference (either prolate-spheroidal or cylindrical) (16), where angles are referenced with respect to the cardiac long-axis and short-axes. This has not been carried out for the first part of this analysis where the angle reported is the angle between the in-plane sheet and x-axis of the image Cartesian reference frame. This is because the first objective has been the qualitative and quantitative comparison of images, which can be more directly achieved within the Cartesian coordinate system. The comparison of

Fig. 2. Three-dimensional visualization of myolaminar architecture from a rat heart (MRI dataset C1LR, 50 × 50 × 50 μm). In the MRI images, in contrast with Fig. 1, the cleavage planes appear brighter than the myolaminae. The atria have been removed by manual segmentation. A: exterior of heart, left/lateral view. PA, root of pulmonary artery/infundibulum of the right ventricle. B: the same view of the ventricles as in A, with the lateral left ventricle (LV) wall and heart base cropped. The 3-dimensional branching network of the myolaminae can be seen on the short- and long-axis cut surfaces. (myolaminae: red; cleavage planes: white). C: magnified view of the anterior myocardium from B (region in box). Greater detail of 3-dimensional myolaminar branching can be seen.
MR images in slices aligned to the cardiac long-axes facilitates conversion of angles between coordinate systems. The angle reported in Fig. 9 is the β-sheet angle reported relative to the epicardial tangent orientation and changing sign depending on position with respect to the left-ventricular centroid (as in Ref. 16).

Quantitative comparison of sheet morphology and orientation from MRI and histology and statistical approach. Initial Registration of the histological and MRI three-dimensional images was carried out manually using the ParaView (Kitware) visualization software by optimally aligning the myocardial blood vessels and the ventricular walls. To achieve more accurate registration and to minimize freezing and sectioning artifacts in the histological images (foldng, compression, and splitting) after manual alignment, we used a deformable registration tool implemented in the ImageJ environment (28). Directional statistics (specifically axial circular statistics) were used for all analyses of laminar orientation angle. Data were plotted to determine their distribution (whether unimodal/bimodal/multimodal). Next, appropriate measures of central tendency and dispersion were selected based on the data distributions. A nonparametric circular-circular correlation test was used to test for association, specifically the circular-circular rank correlation coefficient (25), where a positive value of \( r_0 \) (\( R^2 \)) indicates positive dependence and a low \( P \) value indicates dependence. Statistical analysis and testing was carried out using the Circular Statistics Toolbox (4) for MatLab (Mathworks), custom written code, and the PAST statistics package (University of Oslo) (17).

RESULTS

Visualization of sheets in the cardiac volume. Myolaminar structure is readily observed on the cut myocardial surface of the unfixed viable perfused heart (Fig. 1), and indeed, the myolaminae can be seen to slide during myocardial contraction (movie associated with Fig. 1). However, the demonstration of cardiac sheets requires three-dimensional visualization since two-dimensional images do not distinguish these laminae from tubular bundles of fibers. The three-dimensional continuity of laminar structure in a selected region is shown in Fig. 2 and is further demonstrated in Fig. 3 and in the related movie of the progression through an MRI short-axis stack (C1[HBR]) (25 × 25 × 39 \( \mu \)m). It can be seen in both Fig. 3 and the movie that laminar organization is smoothly continuous throughout most of the myocardium, with gradual transmural change in orientation, but that there are also regions were groups of laminae intersect at \( \sim 90^\circ \). This is explored in detail below. It has long been known that the myocardium has a regular change in fiber orientation from endocardium to epicardium. This regular change must therefore coexist with the intersections of the sheets of unlike orientation (so-called positive and negative sheets).

Qualitative and quantitative validation of the MRI laminar imaging against histology. To demonstrate that the contrast which MRI is measuring is the same laminar structural feature that is described in the classical histological studies (24), we measured laminar orientation in histological sections after MRI (in image data C1[HBR]). Perfusion with Gd-DTPA emphasizes not only myolaminae, but also blood vessels and the profuse network of small capillaries. This is demonstrated in Fig. 3 where blood vessels can be seen as diagonal bright lines in the cross section of the papillary muscle. Blood vessels can therefore look similar to cleavage planes. The three-dimensional laminar continuation of bright structures in the selected two-dimensional images was therefore confirmed by visual inspection before quantitative analysis. The same laminar architecture is seen in the MRI and in selected histological slices. Likewise, the laminar orientation measured by the structure tensor method follows the same spatial distribution (Fig. 4, A and B). Angular difference maps for the two selected histology sections (Fig. 4, A and B) show that throughout most of the long-axis slices the angular difference is below 25°. The pooled orientation measurements from histology were plotted against the pooled MRI orientation (Fig. 5A); a clear correlation is seen. The correlation plot also shows some poorly correlated values, as was observed in the images. The plotting of the correlation of images on Cartesian axes is limited by the continuity of the circular scale through the \( -90^\circ \) to \( +90^\circ \) range. The distributions are therefore shown as rose diagrams (circular histograms where the bars are replaced with sectors) (25). Rose diagrams of the pooled MRI and histology orientation data from three long-axis slices are shown in Fig. 5B. The circular-circular rank correlation coefficient \( r_0 \) is 0.35 with \( P < 10^{-5} \), indicating a high confidence in a moderate to strong positive correlation. Although there are differences in the
distributions, the overall pattern of dominant positive sheet angles in the $+33^\circ$ to $+66^\circ$ range is shared.

Transmural in-plane sheet angle orientation profiles from histology and MRI were compared to further demonstrate the correlation (Fig. 6). Transverse transmural profiles were analyzed for slice 1 and longitudinal profiles for slice 2. Transmural profiles show the same overall properties as described for the whole slices: there is close association between histology and MRI determined laminar orientation, except in some localized regions of high disparity. For slice 1 $r_0$ is in the range 0.17 to 0.33, with $P < 10^{-3}$, and for slice 2 $r_0$ is in the range 0.12 to 0.67, with $P < 10^{-5}$.

Consistency of laminar organization between hearts. The consistency of laminar structure in rat myocardium is demonstrated by comparing MRI determined laminar orientation from four hearts (imaged at $50 \times 50 \times 50 \, \mu m$, referred to as C4LR). Due to differences in cardiac geometry, it is necessary to carry out deformable registration so that laminar architecture can be compared in corresponding myocardial locations. To demonstrate the validity of this approach the comparative analysis was performed in two phases. First, the four hearts were registered without deformation (affine registration only was performed) to morph the C2, C3, and C4 cardiac geometries to the geometry of C1. These affine registered images are referred to as aC2LR, aC3LR, and aC4LR. In a second approach, deformable registration is used to morph the C2, C3, and C4 cardiac geometries to the geometry of C1, and these deformably registered images are referred to as dC2LR, dC3LR, and dC4LR. Images of two long-axis cardiac planes after affine registration and after deformable registration are shown in Fig. 7. Laminar orientation is shown to be qualitatively similar between C1LR, dC2LR, dC3LR, and dC4LR in selected long-axis slices, and comparison of these data with the affine registered data (C1LR, aC2LR, aC3LR, and aC4LR in Fig. 7) qualitatively demonstrates similarity and validates the deformable registration approach.

Quantitative comparison was carried out for the same selected long-axis slices for C1LR, dC2LR, dC3LR, and dC4LR (Fig. 8). The analyses are similar to those carried out to compare the histology and C1HR in Fig. 4, except that in this analysis, the selected long-axis sections were aligned with the cardiac long-axis (in the MRI/histology comparison in Fig. 4, the histological sectioning plane determined the analyzed MRI...
plane). In both selected slices the hearts show similar spatial distribution of in-plane sheet angles, with broadly similar angle distributions (see rose diagrams). There are disparities between the measured angles in some regions, which are shown in the angle difference maps. The dispersion of the orientation distributions is less for the sharper MR images (C1LR) and is greater for the deformably registered images (dC2LR, dC3LR, and dC4LR). Blurring is a consequence of the deformable registration as can be seen by comparing affine and deformable registration in Fig. 7.

The quantification and comparison of transmural sheet angle profiles in C1LR and dC2–d4LR is shown in Fig. 9. This was carried out on the near-coronal long-axis slice in Fig. 7 (slice 45°). In Fig. 9 the B’-sheet angle is shown; sheets with angle of orientation between the cardiac short-axis (0°) and the basally directed LV centroid (+90°) are defined as having positive sheet-angle, and sheets with angle of orientation between the cardiac short-axis (0°) and the apically directed LV centroid (−90°) are defined as having negative sheet-angle.

The sheet angles are measured with respect to a transmural radius normal to the epicardial surface tangent. Transmural profiles show sheet angles across the full range of −90° to +90°. Some profiles show gradual transmural change in the in-plane sheet orientation (Fig. 9, LV anterior left-free wall, apex), some show a sharper change in orientation (Fig. 9, LV posterior right-free wall, equator, and LV anterior left-free wall, base), and some show concertina transmural switching of positive and negative orientation (Fig. 9, LV anterior left-free wall, equator). There are regions of high similarity between the measured transmural profiles in the four hearts (e.g., Fig. 9, LV posterior right-free wall, apex, subendocardium), and there are regions of greater variance (e.g., Fig. 9, LV anterior left-free wall, equator). There are regions of high similarity between the measured transmural profiles in the four hearts (e.g., Fig. 9, LV posterior right-free wall, apex, subendocardium). This is consistent with results presented in the previous figures, where regions of disparity between the sheet orientation in the images have been described.

Manual tracking of myolaminae in the lateral left ventricle. To investigate the nature of the transmural three-dimensional
branching and interconnectivity of myolaminar architecture we manually tracked cleavage planes within C1HR. A midwall starting point (shown as an asterisk in Fig. 10) was chosen, and the three-dimensional continuation of the local cleavage planes and laminae were traced using the Seg3D software. Branching was frequently observed along the three-dimensional continuation of the cleavage planes, and the branching frequently resulted in equally favorable paths. An example of this is shown by the arrow head in Fig. 10. Tracking was stopped when the endocardium was reached, or when sheet structure was no longer discernable (toward the epicardium). A three-dimensionally branching orthotropic structure therefore resulted. This can be seen in the cut-away images. Both the myolaminae and the cleavage planes branch in this manner, being structurally complementary to each other. It is important to note that the branching of the myolaminae continues in three dimensions at the boundaries of the smooth surfaced structure shown in Fig. 10; these boundaries are for illustrative purposes, and no such smooth boundaries separate subnetworks of laminae from the bulk of the myocardium.

**DISCUSSION**

We have shown that whole ventricle myocardial laminar structure can be readily imaged using contrast-enhanced MRI and that the imaged myolaminar architecture is consistent with histology and is consistent between rat hearts.

**Advantages of MRI for imaging of myolaminar structure and future applications.** Using MRI for imaging laminar structure has fundamental advantages over histological methods. Histology is a destructive method, whereas MRI is not, and because of the three-dimensional nature of the MRI it allows structural questions to be answered that cannot be addressed in the presentation of selected data from two-dimensional conventional histological sections. High-spatial resolution MRI has been applied to the beating heart to generate two-dimensional images (23), whereas histology cannot be applied to living tissue. In principle, contrast-enhanced high resolution MRI could be applied to image the movement and reorganization of cardiac laminae between systole and diastole, particularly if applied alongside contrast agents targeted to connective tissue (19). The ability to acquire detailed three-dimensional images of myocardial structure will have important future implications for basic and clinical cardiac research. Ex vivo imaging of unfixed hearts could be used to explore myolaminar reorganization during myocardial thickening and torsion that accompanies contraction. This fuller understanding of laminar sliding may lead to better models of cardiac mechanics (which do not currently incorporate laminar sliding) and a more complete
understanding of the pathophysiology of heart failure, specifically of cardiac interstitial fibrosis and accompanying reduced cardiac compliance. As sheets influence the spread of activation, imaging of sheet changes in disease will provide insights into the structural mechanisms of the initiation and propagation of arrhythmia. There are significant technological hurdles to overcome before these applications are practicable.

The fundamental nature of myolaminae: localized or continuous laminar structure. In early studies myolaminae were described as radial transmural structures, which branched in some regions of the heart (24), and it was proposed that myocardial structure was orthotropic (described fully by a fiber direction, in-sheet direction, and sheet normal direction) rather than uniaxial anisotropic (described fully by a fiber orientation alone, like skeletal muscle). Others have more recently described myocardium as having numerous short gaps of varying dimensions, orientation, and location (13). The debate is therefore currently focused on the extent of the myolaminae and the nature of their branching. The findings of this study are that there is orthotropic structure throughout the left ventricular myocardium (Fig. 2 and movie associated with Fig. 3), except in the immediate subepicardium. The absence of myolaminae in the rat subepicardium is a qualitative observation from the MRI volumes and demonstrated in the movie associated with Fig. 3 and selected MRI long-axis slices and has been previously described in histology images (27). Although orthotropic structure is near-universally present, laminae are observed to be highly branching, and indeed they can be seen to be branching in any selected viewing plane. As demonstrated in Fig. 10 manual tracking of the myolaminae starting from a localized midwall point in the myocardium produces a branched orthotropic network, which becomes several laminae thick toward the epicardium and endocardium. The structures produced resemble the propeller shapes described classically.

Fig. 8. Quantitative comparison of in-plane sheet angle between deformably registered MR images (C1LR and dC2LR, dC4LR). The selected long-axis slices are those described in Fig. 7. A: orientation (top row) maps for a selected near coronal long-axis slice (45°→) for the 4 hearts. Below this (second row) are the corresponding angular differences with comparison to C1LR. The r and P values for each pair-wise correlation to C1LR are shown. In the bottom row are the rose diagrams of the in-plane sheet angle distributions. B is laid out as in A, with a second selected long-axis slice (90°→).
Laminar structure is therefore an orthotropic branching mesh, with the fiber orientation evolving smoothly over space with the laminar orientation behaving likewise, except at localized regions (as discussed below).

Regions of observed differences between laminar orientations. In the comparison of MRI and histological laminar orientation there are regions were the angular difference is high (Fig. 4). In general these regions can be seen in the images to correspond to areas of: 1) low MRI sheet contrast; 2) sectioning artifact in the histology images; and 3) the meeting of positively and negatively orientated in-plane sheets. As such it is likely that these differences are a consequence of imaging artifact, imperfect sectioning, and registration error, rather than any difference between structural features imaged by the two methods. Likewise in the comparison of MR images from the four rat hearts there are regions were the angular difference is high (Fig. 8). Examination of the MRI volumes showed these to correspond to regions of low MRI sheet contrast, the meeting of positively and negatively orientated in-plane sheets; and
tissue would have been subject to edema and degradation. Spatial resolution images of the whole ventricles and unfixed preparations have imaged ex vivo perfusion fixed hearts. We fixed the hearts before imaging to ensure that they were fully differentiated from variability in ex vivo preparation. An implication is that in vivo interheart variability cannot be fully quantified.

**Fig. 10.** The 3-dimensional structure of adjacent myolaminae. Cleavage planes and myolaminae were manually tracked within C1H1R starting from point (1), and the resultant structure was visualized. From the starting point the most obvious 3-dimensional continuation of the laminae was followed. At many points throughout the tracking there were equally favorable paths (as indicated by the arrow head on C). A 3-dimensionally branching orthotropic structure therefore results, as can be seen in A and the cut-away images B and C. Both the myolaminae and the cleavage planes branch in this manner, being structurally complementary to each other. Note that the branching of the myolaminae continues in 3 dimensions and the boundaries of the structure shown are for illustrative purposes.

Observations show that the myolaminae are more distinct in our study than in these studies, but it may be because we do not embed the heart in a gel medium. Limitations of laminar tracking to build a map of simplified model laminar architecture. Having acquired high-resolution MR images of detailed laminar structure (up to 25 × 25 × 37 μm) in the entire ventricle we looked within the structure for evidence of higher-order architecture (band architecture) as has been described previously (6). Local aggregations of sheet structure were tracked manually, and we found that the tracked path was dependent on the approach taken and was subjective. Specifically the manually tracked path depended upon: 1) the starting point within the structure; 2) the anatomical section in which the structure was primarily examined (sagittal/coronal/axial); and 3) the subjective assessment of the operator. Although the latter two considerations would not apply to automated tracking methods, the former consideration will still apply, and we have not investigated this approach further. The image data in the movie associated with Fig. 3 is available for others to do so. Our conclusion from manual tracking is that cardiac laminar structure is highly complex and has many regions of localized structural complexity that are observed at the same sites in the different hearts examined. These localized regions of complex laminar structure are the abutment of laminae of nonsimilar orientation (so called positive and negative sheets). It is not possible to label unique laminae and unequivocally determine their dimensions since they form a densely branching three-dimensional network. This is in agreement with findings we have published previously from DT-MRI (16), where we reviewed evidence for laminar structure as a three-dimensional spatial network. Simplified structural models are sought since these would allow cardiac electromechanics to be integrated and understood. Our findings suggest that the complexity of fiber/sheet structure, combined with the three-dimensional merging and mixing of sheet populations, significantly limits the utility of these simplified structural models, and instead detailed electromechanical computational models incorporating high resolution image data will be required.

**Models of cardiac structure in context with sheet architecture as revealed by MRI.** Recent debate about cardiac structure has focused on three models: 1) the orthotropic myolaminar structure during imaging, and we quantified structure against histology, which requires fixed tissue. There will be structural changes associated with fixation. It is thought that cleavage planes between myolaminae are potential spaces in vivo and that postmortem changes and tissue processing can open up these cleavage planes through dehydration and through the shrinkage of muscle cells that can accompany fixation. It is likely that these postmortem changes have facilitated the detailed high-contrast imaging of laminae and cleavage planes. Although postmortem change may open up laminar structure, we have shown that myolaminar structure is readily observed on the cut myocardial surface of the unfixed viable perfused heart (Fig. 1), and indeed, the myolaminae can be seen to slide during myocardial contraction (movie associated with Fig. 1). Others have shown highly similar laminar structure in two-dimensional imaging of the viable perfused rat heart in mid-diastole (23). In other high-spatial resolution MRI studies, which have used similar tissue preparation (Langendorff perfusion with fixative and Gd-DTPA contrast), myolaminae are visible but less demarcated (5, 7). It is not known why laminae are more distinct in our study than in these studies, but it may be because we do not embed the heart in a gel medium.
model (24; 2) the Functional Syncytial Mesh model (FSM) (2); and the J) Helical Ventricular Myocardial Band (HVMB) model (6). The results for the imaging presented here strongly support generalized orthotropic structure, except in the immediate subepicardium, as described previously (27). We have demonstrated that the laminae exist as a densely branching network or mesh, and as such our results are compatible with the FSM model. However, we believe that this model has underplayed the universality and importance of laminar orthotropy. We have demonstrated at a spatial resolution of 25 \( \times \) 25 \( \times \) 37 \( \mu \)m that the branching laminar structure is continuous in three dimensions. There are regions of abrupt change of myolaminar organization (Figs. 1–4, 6–9, and movies), which are prominent in the basal LV. As such it is possible that the dissection upon which the HVMB model is based follows these regions of positive and negative laminar intersection (as discussed elsewhere in Ref. 16). Nonetheless, the three-dimensional branching of the laminae across these regions of abrupt change of myolaminar orientation, and the continuous fiber orientation change across these regions (33) does not support the HVMB as a useful or appropriate model of cardiac structure or mechanics. Through applying our novel contrast-enhanced imaging approach and image analysis we have shown that MRI can be used to image the laminar architecture of ex vivo hearts in three dimensions; the images produced are qualitatively and quantitatively comparable with histology; 3) the method is repeatable between hearts. Furthermore, using this imaging method we have demonstrated that in the rat laminar architecture is consistent between hearts; confirmed that myolaminae are absent from much of the sub-epicardium; and demonstrated that although localized orthotropy is present throughout the myocardium, tracked myolaminae are branching structures and do not have a discrete identity.

Summary. We hypothesized that it should be possible to image and visualize whole ventricular three-dimensional laminar architecture after contrast-enhanced MRI. Our approach was based on cardiac fixation using Gd-DTPA perfusion in formalin, which allowed us to generate high-contrast MR images of whole ventricle myolaminae and cleavage planes. We visualized these MRI volumes to demonstrate laminar architecture and quantitatively demonstrated that the structural features observed are the same as those imaged in histology. We showed qualitatively and quantitatively that laminar architecture was similar in four rat hearts. Our study supports a model of myocardium as an orthotropic mesh of highly branch-ting laminae.

Limitations. All imaging in this study was carried out on the rat heart, and direct conclusions can only be made about the rat heart. However, laminar structure is known to have grossly similar features in all mammalian species thus far studied (reviewed elsewhere in Ref. 16). Histology involves sectioning, which can introduce image distortions, as can image registration. Statistical correlation is powerful at finding relationships between data, but is not a test of similarity, and circular correlation coefficients and \( P \) values must be interpreted alongside examination of the distribution of the circular variables. Three-dimensional laminar structure was quantified and compared between hearts using two-dimensional quantitative orientation analysis of the in-plane myolaminae, and the analysis of the three-dimensional branching of myolaminae was qualitative. Systematic quantitative three-dimensional analysis of the branching of the myolaminae has not been performed in this study. The histology sections were deformably registered to the MRI, and the degree of distortion of the myolaminar orientation measurements resulting from this approach has not been quantified.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


REFERENCES

H298 QUANTIFICATION OF CARDIAC LAMINAR STRUCTURE FROM MRI


