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High-Resolution *Ex Vivo* Microstructural MRI After Restoring Ventricular Geometry via 3D Printing

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Abstract

Computational modeling of the heart requires accurately incorporating both gross anatomical detail and local microstructural information. Together, these provide the necessary data to build 3D meshes for simulation of cardiac mechanics and electrophysiology. Recent MRI advances make it possible to measure detailed heart motion *in vivo*, but *in vivo* microstructural imaging of the heart remains challenging. Consequently, the most detailed measurements of microstructural organization and microanatomical infarct details are obtained *ex vivo*. The objective of this work was to develop and evaluate a new method for restoring *ex vivo* ventricular geometry to match the *in vivo* configuration. This approach aids the integration of high-resolution *ex vivo* microstructural information with *in vivo* motion measurements. The method uses *in vivo* cine imaging to generate surface meshes, then creates a 3D printed left ventricular (LV) blood pool cast and a pericardial mold to restore the *ex vivo* cardiac geometry to a mid-diastasis reference configuration. The method was evaluated in healthy ($N = 7$) and infarcted ($N = 3$) swine. Dice similarity coefficients were calculated between *in vivo* and *ex vivo* images for the LV cavity (0.93 ± 0.01), right ventricle (RV) cavity (0.80 ± 0.05), and the myocardium (0.72 ± 0.04). The R^2 coefficient between *in vivo* and *ex vivo* LV and RV cavity volumes were 0.95 and 0.91, respectively. These results suggest that this method adequately restores *ex vivo* geometry to match *in vivo* geometry. This approach permits a more precise incorporation of high-resolution *ex vivo* anatomical and microstructural data into computational models that use *in vivo* data for simulation of cardiac mechanics and electrophysiology.

Keywords

3D printing; Magnetic resonance imaging; Cardiac electromechanics; Computational modeling

1 Introduction

Computational modeling can be used to better understand the mechanisms of cardiac electromechanics in both healthy and infarcted hearts. Advanced MRI methods enable

quantifying both functional and microstructural properties of the heart for incorporation into computational models. For example, MRI can be used to: (1) measure tissue displacements with the cine DENSE technique, thereby enabling the estimation of cardiac strain [16]; (2) characterize microstructural orientation and compartment shape through diffusion tensor imaging (DTI) [1]; and (3) characterize myocardial infarct microanatomy using late gadolinium enhancement (LGE) [4].

Several groups have recently developed *in vivo* cardiac DTI (cDTI) techniques [1,9] that have already proven valuable for describing microstructural differences in several forms of cardiomyopathy [6]. However, *in vivo* cDTI still presents major challenges, including the relatively large voxel size needed to acquire sufficient signal-to-noise, compensating for bulk cardiac motion in the preferred reference configuration (i.e., mid-diastasis), and exam time constraints. In order to overcome these limitations, high-resolution *ex vivo* cDTI imaging can be used to build subject-specific computational models [7,12].

In addition, *in vivo* LGE images are a proven technique for estimating myocardial infarct volume, transmural, and location. Infarct detection based on LGE, however, is limited by inherently low spatial resolution and the sensitivity to contrast agent dosing and washout. A large voxel size makes quantifying scar volume prone to error, especially in the border zones where low signal intensity in healthy tissue is averaged with high signal intensity due to fibrotic tissue [5]. Although LGE provides diagnostic clinical data, this method is generally inadequate for computational model building. Therefore, one approach is to use high-resolution *ex vivo* LGE to overcome the limitations of low resolution *in vivo* data.

Consequently, while meaningful cine DENSE data can only be acquired *in vivo*, both cardiac DTI and LGE scar imaging are better performed *ex vivo*. There remains, however, an open problem of acquiring *ex vivo* MRI data in hearts that accurately conform to *in vivo* geometry. Current *ex vivo* cDTI methods typically use an injection of rubber silicone to fill the four heart chambers [5,8]. Although this method approximately restores the geometry of the compliant heart, it is very difficult to control cavity shape and volume, which limits conformance of the *ex vivo* data to the *in vivo* data. Post-processing techniques such as elastic registrations are able to partially overcome the mismatch, but can distort the quantitative information stored in the registered image voxels. Indeed, there is no guarantee that the elastic registration correctly accounts for the underlying tissue deformation between the *in vivo* and *ex vivo* configurations. For example, through plane motion and intra-slice torsion cannot be accurately estimated with image-based elastic registration techniques [11] and the resulting warping field may not transform microstructural orientation information accurately. In other organs, 3D printing has proven to be a feasible approach to provide external shape constraints that maintain and enforce geometric accuracy, thereby permitting registration to *in vivo* targets with a simpler rigid registration [15].

Herein, we propose a new method for *ex vivo* heart preparation that uses *in vivo* cardiac MRI to build 3D printed molds. The proposed method: (1) accurately restores *ex vivo* ventricular cardiac geometry to an *in vivo* mid-diastasis reference configuration; and (2) enables rigid registration of high-resolution *ex vivo* anatomical, scar, and cDTI data to the corresponding reference configuration derived from the *in vivo* images.

2 Methods

2.1 Study Design

Healthy (N = 7) and infarcted (N = 3) swine hearts were included in this study. All animal experiments were conducted according to a research protocol approved by the UCLA Institutional Animal Care and Use Committee (ARC protocol # 2015–124). Infarct induction was achieved by fluoroscopic guided injection of microspheres into a secondary branch of the left anterior descending (N = 2) and left circumflex (N = 1) coronary arteries to occlude blood flow. Infarcted subjects were then imaged 6–10 weeks after induction to allow for complete scar formation.

2.2 *In Vivo* Acquisition and 3D Printing of Cardiac Scaffolds

All subjects were imaged *in vivo* using a 3T MRI scanner (Prisma, Siemens) and a 32-channel chest and spine coil. Balanced steady state free precession (bSSFP) 2D cine cardiac scans were pulse-oximetry gated with single breath holds (base matrix, 160×288 ; field of view, $236 \text{ mm} \times 340 \text{ mm}$; spatial resolution, $1.18 \times 1.18 \times 8.0 \text{ mm}^3$; 60 phases per cardiac cycle). For infarct subjects, LGE was performed by injecting a gadolinium-based contrast agent (0.3 ml/kg diluted in 5 ml of saline and injected at 0.5 ml/s) 10 min prior to imaging. A phase sensitive inversion recovery (PSIR) LGE sequence was used to image the entire heart volume (base matrix, 154×256 ; field of view, $255 \text{ mm} \times 340 \text{ mm}$; flip angle, 20° ; Inversion Time = 260 ms; spatial resolution, $1.33 \times 1.33 \times 8.0 \text{ mm}^3$).

Cine images were manually segmented (Horos, v3.1.1) at a mid-diastolic time frame. Masks of the left ventricular (LV) blood pool and pericardium were drawn for all mid-diastolic images. Segmentation time for the entire heart volume for both the LV blood pool and pericardium was approximately 30 min. The LV blood pool and pericardial masks were converted into surface meshes with 3Matics (Materialise NV). Local smoothing was used to connect the short-axis contours corresponding to slices acquired 8 mm apart. Meshes of the pericardial molds and LV blood casts were then imported and printed (Ultimaker 3 extended, Ultimaker) with polylactic acid (PLA). Print times were approximately 45 min for the LV blood pool casts and approximately 210 min for the pericardial molds (Fig. 1).

2.3 *Ex Vivo* Extraction and Acquisition

For infarcted subjects, a double dose of gadolinium (gadobenate dimeglumine or gadopentetic acid, 0.6 ml/kg) was injected intravenously ten minutes prior to euthanasia (following institutional guidelines) to achieve LGE when performing *ex vivo* imaging.

After excision of the heart, the LV blood cast was inserted into the LV, using the papillary muscles and position of the aorta as reference to correctly orient the 3D print. The heart was then placed inside the customized pericardial mold to provide an external boundary for the epicardium (Fig. 1). The right ventricle (RV) was then filled with silicone rubber compound (Polyvinylsiloxane, Microsonic Inc.). The geometrically restored heart was placed in a cylindrical container and filled with a perfluoropolyether solution (Fomblin, Solvay) that produces no MRI signal.

Ex vivo MRI was performed on the same 3T MRI scanner used during the *in vivo* studies. A 15-channel transmit/receive knee coil was used to acquire T1-weighted gradient echo (GRE) images (echo time (TE)/repetition time (TR), 3.15 ms/12 ms; flip angle, 25°; base matrix, 160 × 160; field of view, 160 mm × 160 mm; spatial resolution, 1.0 × 1.0 × 1.0 mm³; averages, 6) and DTI (TE/TR, 62 ms/15560 ms; flip angle, 180°; base matrix, 150 × 150; field of view, 150 mm × 150 mm; spatial resolution, 1.0 × 1.0 × 1.0 mm³; b-values, 0 s/mm² and 1000 s/mm²; number of directions, 30; averages, 5).

2.4 Image Processing and Analysis

The workflow of image processing and analysis included five steps:

1. *Landmark Registration* – *In vivo* images (bSSFP and LGE) were registered to *ex vivo* T1-weighted images at their native voxel resolution using the MITK Workbench [14]. The registration method used landmark-based rigid registration, which allowed for qualitative image overlays between *in vivo* and *ex vivo* images and provided a more accurate basis for the following optimized semi-automatic 3D rigid registration. Landmarks consisted of four points in the most basal slice (center of the septal wall, anterior papillary muscle, center of the free wall, and posterior papillary muscle) and four points in similar locations near the apex.
2. *Diffusion Tensor Reconstruction* – *Ex vivo* DTI tensors were reconstructed using linear least squares. *Ex vivo* high-resolution reconstructed diffusion tensors were overlaid on the registered *in vivo* images and shown as superquadric tensor glyphs [3], which completely characterize the tensor shape and orientation (Fig. 2).
3. *Segmentation* – Manual segmentation of the landmark based *in vivo* and *ex vivo* LV blood pool, RV blood pool, and the myocardial tissue were performed in MITK Workbench and exported as binary masks. The apical and basal boundaries for each segmentation were defined from the *in vivo* cine images.
4. *Semi-Automated Registration* – *In vivo* and *ex vivo* LV blood pool binary images were registered with an automated 3D rigid regular-step gradient descent algorithm (Matlab, MathWorks), which scaled *ex vivo* voxels to match *in vivo* voxels. The transformation generated from this registration was also applied directly to the RV blood pool and the myocardial tissue binary images. The registered binary masks were constructed using the *in vivo* voxel size in order to avoid interpolating data from low to high-resolution.
5. *Quantitative Analysis* – *In vivo* and *ex vivo* registered LV blood pool, RV blood pool, and myocardial tissue binary images were used to compute volumes and Dice similarity coefficients (DSC). LV and RV volumes were also compared between *in vivo* and *ex vivo*. Linear regressions were computed for each of the different measurements displaying the line of best fit and 95% confidence intervals. Pearson's correlation coefficients (R^2) were calculated to determine the strength of correlation between *in vivo* and *ex vivo* volumetric data.

3 Results

Image registration was qualitatively evaluated by overlaying different combinations of acquired data for all subjects. One infarcted subject is shown in Fig. 2. For healthy subjects, native resolution *ex vivo* T1-weighted images and *ex vivo* DTI glyphs were overlaid with *in vivo* bSSFP images at a short-axis mid-ventricular slice location. For infarcted subjects, an additional overlay of the *in vivo* LGE with *in vivo* bSSFP was also displayed to show concordance of the scar tissue with anatomy. In general, the images were all well registered.

DSC for each subject is shown in Table 1. Across all subjects, mean DSC $\pm$ standard deviation (SD) for the LV cavity was 0.93 ± 0.01 , for the RV cavity was 0.80 ± 0.05 , and for the myocardial tissue was 0.72 ± 0.04 . In Fig. 3, Pearson correlation coefficients for linear regressions are $R^2 = 0.95$ and $R^2 = 0.91$ for the LV cavity and RV cavity, respectively.

4 Discussion

Several computational studies of cardiac contraction and electrophysiology incorporate rule-based myofibers [2] into the models to account for cardiac microstructure. However, rule-based myofiber models do not take into account the regional variation of aggregate cardiomyocyte orientation, are not subject specific, and the effect of including rule-based models in lieu of subject specific myofibers has not been thoroughly evaluated. These factors highlight the necessity to build computational models based, when possible, on subject specific microstructural data.

Previous *ex vivo* DTI heart studies used several methods to restore ventricular cavity shape. Teh *et al.* [10] arrested rat hearts with potassium, perfused them with Karnovsky's fixative, and immediately fixed the tissue. Potassium arrest results in a relaxed, diastasis-like state, but is susceptible to tissue shrinkage from the fixative [13] and deformation due to lack of intracavity pressure or surface constraint. Kung *et al.* [5] and Sack *et al.* [8] fixed swine hearts with buffered formalin followed by an injection of rubber silicone compound to fill the four heart chambers. Using this method, the heart cavities may be over-or under-filled since there are no constraints to preserve the *in vivo* anatomy.

Using 3D printed molds to restore the shape of fresh myocardium eliminates tissue shrinkage artifacts, minimally impacts the imaged geometry, and enables high-resolution *ex vivo* LGE imaging. The reported high DSC for the LV cavity across subjects and good DSC for the RV cavity and myocardial tissue, in addition to highly correlated cavity volumes, suggest that using 3D printed scaffolds is an accurate approach to restore *ex vivo* geometry to a reference *in vivo* configuration. Subsequent alignment via rigid registration permits fusing *in vivo* and *ex vivo* data. From here, computational models of the heart can be built directly from the high-resolution *ex vivo* cDTI data in the preferred reference configuration without any additional pre-processing, while also accommodating direct incorporation of well-registered *ex vivo* infarct information and *in vivo* motion data.

One limitation of this approach is the unclear *in vivo* boundaries of the RV cavity and RV tissue during segmentation. While the cavity and tissue contrast is well defined in *ex vivo* scans, *in vivo* RV myocardium and blood pool boundaries are not as clearly defined,

therefore limiting DSC to lower values for both the myocardial tissue and the RV cavity. Our approach also used rigid registration methods. Non-rigid registration methods might provide higher DSC and higher correlations of cavity volumes, but our method allows for microstructure reconstructed from the *ex vivo* DTI to be aligned without non-rigidly deforming the tensor orientation information, which would need to be validated. Another limitation of this approach arises from the relatively short time interval between *in vivo* and *ex vivo* imaging. There were approximately eight hours between the acquisition of *in vivo* cardiac cine MRI and the extraction of the heart. Approximately five of these hours were required for 3D printing the LV blood cast and pericardial mold. The remaining time allowed for only one segmentation, limiting the evaluation of intra- and inter-observer variations in image segmentation. After *in vivo* cine segmentation, the rest of the printing preparation time was used to generate smooth LV blood pool and pericardial surfaces from image contours. Finally, additional time was included into the preparation workflow as a precaution in case of print failure.

Although the results shown here are promising, further work is still needed. Separate segmentations of the LV and RV myocardium would permit computing DSC and tissue volumes comparison between *in vivo* and *ex vivo* LV and RV independently. Due to a lower image contrast in the RV, segmentation of the RV tissue is prone to larger errors and uncertainty, which need to be evaluated. Additionally, segmentation of the *in vivo* and *ex vivo* infarct regions would provide further quantitative measures such as scar DSC and volumes.

This work demonstrates a new method to restore *ex vivo* ventricular geometry to an *in vivo* reference configuration through 3D printing and rigid registration. Quantitative validation through volumetric and DSC comparisons confirms the accuracy of the approach and makes available anatomical and microstructural data more amenable to computational electromechanical modeling studies of healthy and infarcted hearts.

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References

1. Aliotta E, Wu HH, Ennis DB: Convex optimized diffusion encoding (CODE) gradient waveforms for minimum echo time and bulk motion-compensated diffusion-weighted MRI. *Magn. Reson. Med* 77(2), 717–729 (2017) [PubMed: 26900872]
2. Bayer JD, Blake RC, Plank G, Trayanova NA: A novel rule-based algorithm for assigning myocardial fiber orientation to computational heart models. *Ann. Biomed. Eng* 40(10), 2243–2254 (2012) [PubMed: 22648575]
3. Ennis DB, Kindlmann G: Orthogonal tensor invariants and the analysis of diffusion tensor magnetic resonance images. *Magn. Reson. Med. Off. J. Int. Soc. Magn. Reson. Med* 55(1), 136–146 (2006)
4. Kellman P, Arai AE, McVeigh ER, Aletras AH: Phase-sensitive inversion recovery for detecting myocardial infarction using gadolinium-delayed hyperenhancement. *Magn. Reson. Med. Off. J. Int. Soc. Magn. Reson. Med* 47(2), 372–383 (2002)

5. Kung GL, et al.: Microstructural infarct border zone remodeling in the post-infarct swine heart measured by diffusion tensor MRI. *Front. Physiol* 9 (2018)
6. Nielles-Vallespin S, et al.: Assessment of myocardial microstructural dynamics by in vivo diffusion tensor cardiac magnetic resonance. *J. Am. Coll. Cardiol* 69(6), 661–676 (2017) [PubMed: 28183509]
7. Ponnaluri AV, et al.: Electrophysiology of heart failure using a rabbit model: from the failing myocyte to ventricular fibrillation. *PLoS Comput. Biol* 12(6), e1004968 (2016) [PubMed: 27336310]
8. Sack K, et al.: Effect of intra-myocardial Algisyl-LVRTM injectates on fibre structure in porcine heart failure. *J. Mech. Behav. Biomed. Mater* 87, 172–179 (2018) [PubMed: 30071487]
9. Stoeck CT, et al.: Dual-phase cardiac diffusion tensor imaging with strain correction. *PLoS One* 9(9), e107159 (2014) [PubMed: 25191900]
10. Teh I, et al.: Resolving fine cardiac structures in rats with high-resolution diffusion tensor imaging. *Sci. Rep* 6, 30573 (2016) [PubMed: 27466029]
11. Verzhbinsky IA, Magrath P, Aliotta E, Ennis DB, Perotti LE: Time resolved displacement-based registration of in vivo cDTI cardiomyocyte orientations. In: 2018 IEEE 15th International Symposium on Biomedical Imaging (ISBI 2018), pp. 474–478. IEEE (2018)
12. Wang VY, Lam H, Ennis DB, Cowan BR, Young AA, Nash MP: Modelling passive diastolic mechanics with quantitative MRI of cardiac structure and function. *Med. Image Anal* 13(5), 773–784 (2009) [PubMed: 19664952]
13. Wehrl HF, et al.: Assessment of murine brain tissue shrinkage caused by different histological fixatives using magnetic resonance and computed tomography imaging. *Histol. Histopathol* 30(5), 601–613 (2015) [PubMed: 25504583]
14. Wolf I, et al.: The medical imaging interaction toolkit. *Med. Image Anal* 9(6), 594–604 (2005) [PubMed: 15896995]
15. Wu HH, et al.: A system using patient-specific 3D-printed molds to spatially align in vivo MRI with ex vivo MRI and whole-mount histopathology for prostate cancer research. *J. Magn. Reson. Imaging* 49(1), 270–279 (2019) [PubMed: 30069968]
16. Zhong X, Spottiswoode BS, Meyer CH, Kramer CM, Epstein FH: Imaging three-dimensional myocardial mechanics using navigator-gated volumetric spiral cine DENSE MRI. *Magn. Reson. Med* 64(4), 1089–1097 (2010) [PubMed: 20574967]

Fig. 1.

Workflow for 3D printing restoration of ventricular geometry. *In vivo* images were acquired followed by manual left ventricle (LV) and pericardial segmentation. Segmentations were then converted to surface meshes and 3D printed. The LV blood pool cast was inserted into the excised heart, the heart was placed into the pericardial mold, and the right ventricle (RV) was filled with a rubber silicone compound. *Ex vivo* images were acquired and rigidly registered with *in vivo* images.

Fig. 2.

Image overlays of a mid-ventricular slice of an infarcted swine heart with a magnified view of the infarcted tissue. *In vivo* cine images were registered with *in vivo* LGE (left), *ex vivo* LGE (middle), and *ex vivo* cDTI tensor glyphs (right). The epicardial and endocardial boundaries between healthy and infarcted tissue agree well in each images. The manual segmentation (white contour) of the infarct as seen on the *ex vivo* LGE is superimposed on the *ex vivo* DTI.

Fig. 3. Scatter plots of *in vivo* and *ex vivo* LV and RV cavity volumes showing both healthy hearts (o) and infarct subjects (+). Linear regression fits and 95% confidence interval (CI) are reported.

Table 1.

DSC for the LV cavity, RV cavity, and myocardial tissue in all subjects.

Heart #	Condition	LV cavity	RV cavity	Myocardium
1	Healthy	0.93	0.75	0.73
2	Healthy	0.94	0.81	0.72
3	Healthy	0.94	0.77	0.72
4	Healthy	0.94	0.79	0.71
5	Healthy	0.94	0.72	0.62
6	Healthy	0.93	0.81	0.75
7	Healthy	0.92	0.81	0.77
8	Infarct	0.90	0.84	0.71
9	Infarct	0.95	0.89	0.78
10	Infarct	0.92	0.83	0.67
mean $\pm$ SD		0.93 $\pm$ 0.01	0.80 $\pm$ 0.05	0.72 $\pm$ 0.04