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Citation

Published Version
doi:10.1007/s10237-012-0461-0

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Stress and strain in load-dependent remodeling of the embryonic left ventricle assessed by finite element modeling

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Running Title:

FE modeling of left ventricular remodeling

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ABSTRACT

Altered pressure in the developing left ventricle (LV) results in altered morphology and tissue material properties. Mechanical stress and strain may play a role in the regulating process. This study showed that confocal microscopy, three-dimensional reconstruction, and finite element analysis can provide a detailed model of stress and strain in the trabeculated embryonic heart. The method was then used to test the hypothesis that end-diastolic strains are normalized after altered loading of the developing LV. Stage-29 chick LV’s subjected to pressure overload and underload were confocal-imaged and reconstructed with full trabecular morphology. Measurements of material properties, intraventricular pressures, and ultrasonic image correlation were used in the analysis and verification. The resulting overloaded models had significantly larger LV volume than control or underloaded. In all models, calculated stress and strain were largest in the trabeculae and lowest in the outer wall. Strain was not normalized in pressure-overloaded models, as significantly greater volume was strained less than in control models. Stress distribution was similar at lower stress levels. Neither stress nor strain was normalized in underloaded models. The results may help explain features of cardiovascular morphogenesis and aid in selecting strain values for manipulating cultured cells or tissue-engineering constructs.

Keywords: chick embryo, heart development, finite element analysis

INTRODUCTION

The heart develops in a mechanically active environment, supplying blood to the developing embryo while simultaneously undergoing growth, morphogenesis, and functional maturation. In the early embryonic period, the heart changes from a smooth-walled, muscle-wrapped tube through a trabeculated phase to the mature four-chambered form. Both genetic and epigenetic factors regulate this process; one of the principal epigenetic factors is mechanical load.
The chick embryo is a well-established model for studying the effects of altered mechanical load on the developing heart. The model enables direct intervention and visualization and its developmental process is comparable to that of mammalian embryos. Experimental paradigms altering regional embryonic left ventricular (LV) pressure have demonstrated that load regulates cardiac development. Conotruncal banding (CTB) increases both diastolic and systolic intraventricular pressure.\textsuperscript{3, 15} Chick hearts subjected to CTB at Hamburger and Hamilton stage 21,\textsuperscript{12} or embryonic-day (ED) 3.5, show myocyte hyperplasia;\textsuperscript{3} ventricular chamber dilatation, thickening of the compact myocardium and trabeculae, and spiraling of the trabecular course;\textsuperscript{30} acceleration of the developmental change in transmural myofiber angle distribution;\textsuperscript{34} significantly stiffer stress-strain properties;\textsuperscript{22} and precocious development of the His-Purkinje system.\textsuperscript{27}

Decreased hemodynamic load produced by chronic verapamil suffusion to the extraembryonic vascular bed from stage 21 decreases diastolic and systolic LV pressure, dorsal aortic blood flow, stroke volume, and ventricular mass.\textsuperscript{4} These hearts have thinner compact myocardium and a higher proportion of trabeculae.\textsuperscript{29} Decreased hemodynamic pressure produced by left atrial ligation (LAL)\textsuperscript{28} at stage 21 or 24 results in altered strain relationships;\textsuperscript{35, 36} delay in His-Purkinje development,\textsuperscript{27} and hypoplastic left heart syndrome (HLHS).\textsuperscript{6, 30}

Abundant evidence thus suggests that in the embryo, as in the mature heart, myocardial elements sense mechanical loads and translate them into some nature of biochemical signals that result in increased or decreased growth involving modified cellular and/or extracellular structure. The passive stiffening and hyperplasia with increased mechanical load suggest that mechanical strain might be both a triggering and controlling factor, since a larger and stiffer myocardium would tend to compensate for the increased pressure. A first step in testing this hypothesis is comparison of LV strain between normal hearts and those subjected to altered loading. Strain has been measured in the mature heart by methods such as cine-radiography of implanted metal...
beads\textsuperscript{7} and high-resolution MRI tagging.\textsuperscript{24} The resolution of these techniques is not sufficient for the early embryonic heart. In the embryo, estimation of epicardial strains by video tracing of surface markers\textsuperscript{36} and 2D echocardiography\textsuperscript{5} is possible, but neither measure strain throughout the volume. Numerical methods can supplement these limited techniques by calculating stress and strain throughout the volume, if estimates of local tissue material properties and a detailed three-dimensional model of the geometry are available.

One goal of this work is to show that confocal microscopy, three-dimensional reconstruction, and finite element analysis can provide a detailed model of stress and strain in the trabeculated embryonic heart. Finite element models of the embryonic heart with full trabecular geometry do not currently exist. We then use this method to test the hypothesis that the changes in morphology and mechanical properties produced by altered internal pressure in the embryonic chick heart normalize tissue strain to control levels. Both increased pressure, created by conotruncal banding, and decreased pressure, created by verapamil suffusion, are studied to test whether the mechanism is bidirectional. Confocal images of the LV in the passive unloaded state are digitally reconstructed and subjected to nonlinear finite element stress-strain analysis using experimentally measured end-diastolic pressure and passive hyperelastic stress-strain relationships. The resulting magnitude and distribution of stress and strain are then compared between experimental and control groups.

**MATERIALS AND METHODS**

*Pressure Overload and Control*

Fertilized white Leghorn chicken eggs were incubated blunt end upward at 38.5\textdegree{} C and 62\% humidity in a forced-downdraft incubator to stage 21. Under a dissecting microscope, access to the embryo was obtained by opening the shell and incising a small region of the outer
and inner shell membranes. A loop of 10/0 nylon suture was tied in a secure but non-binding manner around the outflow track of the developing heart. Embryos with signs of malformation or bleeding were discarded. The opening in the shell was sealed with parafilm and the egg re-incubated until stage 29. The shell was opened and re-sealed with parafilm in matched controls.

**Pressure Underload**

Pressure underload was created by chronic verapamil suffusion. Eggs were incubated to stage 21, when the embryo was exposed by opening the shell and removing the adjacent inner shell membrane. One end of primed PE-60 polyethylene tubing was positioned on the surface of the extraembryonic vascular bed and the other attached to the flow modulator of an Alzet miniosmotic pump (200 µl reservoir, Alza, Palo Alto, CA). The pump had a constant flow rate of 1 µl/h to suffuse the vascular bed with verapamil at a rate of 1 ng/h. The window in the shell was covered with parafilm, and the eggs were returned to the incubator. The vascular bed was suffused with saline in matched controls.

**Pressure measurement**

LV pressure was measured with a servo-null pressure system (model 900A, World Precision Instruments, Sarasota, FL) on a parallel group of stage-29 embryos prepared as described above. A 7-µm, fluid-filled, glass pipette positioned by micromanipulator (Leitz, Wetzlar, Germany) punctured the LV. Accuracy, linearity, and frequency response of the system have been previously determined. A custom LABVIEW software program (National Instruments, Houston, TX) recorded 10-30 pressure cycles per heart. Intravascular pressure was the difference between measured pressure and pressure recorded from immersing the tip in the extraembryonic fluid at the same level as the ventricle. Study size was n=14 control, n=10 overloaded, n=13 underloaded, and n=12 saline control.
Confocal imaging

Embryos were harvested at stage 29 and heparin administered to inhibit clotting. Ice-cold chick cardioplegia solution\textsuperscript{31} perfused through the atria arrested the ventricle in the passive, unloaded state and removed blood. Hearts were then perfused with 4\% paraformaldehyde for fixation (one week at 4 °C) and to enhance tissue autofluorescence. After PBS washing, hearts were dissected out and pinned dorsal side up in a Sylgard imaging chamber on the microscope stage. Two changes of a 50\%/50\% mixture of benzyl alcohol:benzyl benzoate dehydrated and rendered the specimens transparent.\textsuperscript{21} This process allows laser penetration through the LV depth to resolve trabecular geometry without physical sectioning.

The hearts were imaged whole-mount on a Leica SP2 TCS AOBS upright confocal microscope (Fig. 1) in 3-\(\mu\)m steps in the dorsoventral direction (z). The field of the 10X 0.3 NA dry objective necessitated 3 or 4 stacks in the xy directions. Images were saved as uncompressed TIFF files at 8 bits per channel. The study population was n=9 control, n=7 pressure overloaded, n=7 pressure underloaded, and n=7 saline control.

Three-dimensional Reconstruction.

Raw images containing 512x512 pixels in z-stacks of 500-600 slices were imported into the Amira program (Visage Imaging GmbH, Berlin). Images were merged in the x-y directions to create a single stack (Fig. 1). Correction was made for isotropic shrinkage of 19\% from the ethanol dehydration, as determined previously.\textsuperscript{21} The merged images were downsampled from 3-\(\mu\)m to 6-\(\mu\)m cubic voxels, as this was sufficient to resolve the geometric features.

In each slice, the LV was traced with interactive pen to remove the atria, right ventricle, and valves (Fig. 1). The entire interventricular septum remained. Studies using ellipsoidal solid models of the heart with various wall thicknesses and added material to mimic valves or septal
material showed that removing the valves had a negligible effect on wall stress. Thresholding segmented the grayscale images into background and tissue following a study of percentage change in volume by varying the lower threshold from 20 to 60. The threshold needed to be adjusted on each specimen and manual scanning through the stacks was often necessary.

Total tissue volume and cavity volume were calculated for the reconstructions for each of the models using the Amira Segmentation Editor and Tissue Statistics module. Volumes were compared with one-way ANOVA and Tukey post-hoc test.

Removing a small amount of the superior material created a smooth surface for application of boundary conditions. Finally, Amira built-in functions reconstructed the surface with triangular surface elements.

*Finite Element Pre-Processing: Mesh, Material Properties, and Boundary Conditions*

The surface meshes produced by Amira were imported into HyperWorks software (Altair Engineering, Inc., Troy, MI) for pre-processing. Hypermesh created solid meshes averaging 450,000 four-node tetrahedral elements (tet4) (Fig. 2). There were on average 5-8 elements throughout the wall and 4-10 elements in the cross-section of the trabeculae.

The material description was obtained from an LS-DYNA feature that optimizes material parameters for a best match of finite element results to user-specified experimental stress-strain measurements. The finite element model was a 10x10x20-μm bar meshed with 1000 elements and loaded in uniaxial tension. Experimental results for control and pressure-overloaded LV tissue were from results of Miller et al. Stress-strain results for pressure-underloaded and saline control ventricles were measured for this study by an identical procedure from embryos treated at stage 21 as described here (Fig. 3). An isotropic Mooney-Rivlin material gave a less-than-optimal match, but a 4th-order isotropic Ogden model gave excellent agreement. The strain energy per unit volume $W$ for the Ogden model is
\[ W = \sum_{j=1}^{4} \frac{\mu_j}{\alpha_j} (\lambda_1^{\alpha_j} + \lambda_2^{\alpha_j} + \lambda_3^{\alpha_j} - 3) + 0.5K(J - 1)^2, \]

where \( \lambda_i \) are the principal stretch ratios, \( \mu_j \) and \( \alpha_j \) are material parameters, \( J \) is the relative volume, and \( K \) is the bulk modulus. Table 1 gives the parameters. Poisson’s ratio was 0.49.

Models were loaded by the measured internal end-diastolic pressure (62.5 Pa control, 93.3 Pa overloaded, 41.3 Pa underloaded) using the HyperWorks masking function. The outside surfaces were load-free. Nodes on the leveled portion of the upper surface of the LV models were fixed in 3 translational degrees of freedom.

### Finite element analysis

A four-node tetrahedral element, tet4, was used in LS-DYNA; a ten-node tetrahedral element (tet10) is available but it gave inaccurate results on test volumes. An explicit solution scheme reduced the core storage requirements. The pressure load increased linearly from 0 to 1 sec with automatic time-stepping. The accuracy of the explicit method was tested on a simplified heart model of 32,000 face elements. Solutions from explicit and implicit schemes differed by approximately 1%.

In a convergence study through successive mesh refinement with the full Ogden model and 117K, 277K, 307K, and 450K solid tetrahedral elements, run time increased approximately exponentially with number of elements. Exact doubling of the number of elements was not possible because of the need to reface in Amira. Strains converged to within 2% at the finest mesh refinement. The resulting LS-DYNA explicit analysis required from 5 to 21h of elapsed time on desktop computer, with a mean of approximately 8 h.

### Finite element post-processing

Three principal stresses and von Mises (VM) stresses were displayed graphically on the 3D volume in HyperWorks. To enable quantitative comparison, we used HyperWorks to read the
LS-DYNA results and create tables of element number, volume, stress, and strain (principal and VM) for each of the models. These tables were then input to a custom MATLAB program which calculated histograms of LV volume, both percentage and absolute, versus stress and strain levels. Stress was binned in 50 Pa increments and strain in 1% increments. All statistical comparisons were made with one-way ANOVA and Tukey post-hoc tests.

_Ultrasonic Imaging_

To provide epicardial strain measurements for comparison with calculated values, ultrasonic B-mode and M-mode images of control and pressure-altered stage-29 hearts were obtained with a Vevo 660 ultrasound biomicroscopy system with RM708 scanhead (VisualSonics, Toronto, Canada) by the method of McQuinn et al.\textsuperscript{19} As \textit{in ovo} imaging is limited by the fairly large probe footprint and short fixed focal distance, an \textit{ex ovo} culture setup\textsuperscript{1,37} allowed freer positioning of the scanhead and closer positioning of the embryo to the scanhead. Resolution was 30 \textmu m. Irfanview software (Irfan Skiljan, www.irfanview.com) extracted sequential tiff images from the ultrasound videos. These images were analyzed with a MATLAB program for digital image correlation and tracking\textsuperscript{8} to produce surface plots of displacement and strain.

RESULTS

_Left Ventricular Pressures_

LV pressures were significantly larger after CTB and significantly smaller after verapamil administration. Peak systolic pressures were 4.73±0.16 mmHg in control (mean±SE), 3.70±0.08 (p<0.001) in underloaded, and 7.32±0.29 (p<0.001) in overloaded. End diastolic pressures were 0.47±0.05 in control, 0.31±0.05 in underloaded, and 0.70±0.17 in overloaded.

_Left Ventricular Tissue and Cavity Volumes_

Mean LV tissue volume of the pressure-overloaded group was 51% larger than the control
group (p<0.01), while that of the pressure-underloaded group was not significantly different from control (Table 2). Similarly, the mean LV cavity volume of pressure-overloaded models was significantly larger, 49%, than controls (p<0.05 vs control and underloaded) (Table 2).

**Stress Calculations**

In control models, the calculated VM stress was less than 100 Pa in most regions of the compact wall, with the lowest stresses in the apex (Fig. 4a). Stresses were larger in the trabeculae, 200-300 Pa, with some peaks up to 500 Pa. Pressure-underloaded models had similarly low wall stress (Fig. 4b), with lower trabecular stresses than in control. VM stresses in overloaded models were generally larger than those in control models in all regions (Fig. 4c). More of the trabeculae had VM stresses of 200-300 Pa, with the majority of stress peaks in the trabeculae approaching 500 Pa.

In all models, the histograms of both absolute volume and percentage volume versus stress show exactly the same trends, so only the percentage volume histograms are shown here. The volume-stress relationship of the control group peaks between 50 and 100 Pa and falls monotonically to negligible volume by 300 Pa (Fig. 5). The overloaded histogram peaks slightly higher, 100-150 Pa, and has more tissue at stresses from 150-300 Pa (p<0.05). The underloaded volume-stress histogram has a sharp peak of 54% volume at 50 Pa and a much sharper fall-off by 150 Pa (p<0.05 vs control and overloaded).

Principal stresses were compared to VM stress in a representative model (Fig. 6). First principal stress was tensile, less than 100 Pa in the wall with trabecular peaks to 250 Pa. Third principal stress was tensile or low compressive in the wall, with higher compressive magnitudes in the trabeculae. As expected, locations of large VM stress match those of large principal stresses. In the absence of research on whether tensile or compressive stresses and strains have the greatest effect on remodeling, use of VM stress and strain for analysis seems appropriate.
Strain Calculations

In control models, VM strain was lowest in the apex and the outermost regions of the compact wall, increasing to 7% in wall regions bordering the trabeculae and 17-19% peaks in the trabeculae (Fig. 7a). Compact wall strains were similarly low in pressure-underloaded models (Fig. 7b), with peaks in the trabeculae. Pressure-overloaded models (Fig. 7c), however, had much smaller strains: less than 1% in the apex and outer wall and peaks in the trabeculae to 8%.

The control group volume-strain histogram has a broad global peak from 11-14% (Fig. 8). Note that because stress and strain are not linearly related, the plots for volume-strain are not simple multiples of the plots for volume-stress. The volume-strain histogram for underloaded models is intermediate between control and overloaded and differs significantly from control at strains less than 8% and greater than 11%. The volume-strain relationship for pressure-overloaded models peaks sharply at 3%, with approximately half of the LV tissue strained less than 3% and less than 2% strained more than 10% (p<.05 vs control and underloaded).

Ultrasonic measurements

Surface strains ranged from 2-10% as calculated from a two-dimensional strain map for the imaged portion of the LV epicardial surface. Considerable variability existed in the cyclic repeatability of the speckle pattern which limited the ability to precisely bracket the strains.

Saline control models

All saline control hearts were analyzed in the same manner as the normal controls, overloaded, and underloaded hearts. Their results did not differ from the normal controls in any aspect: volume, stress-strain relationship, and calculated stresses and strains. For clarity, specific results are not shown here.
DISCUSSION

This study used techniques in confocal microscopy, digital reconstruction, and finite element analysis, along with pressure measurement, material property characterization, and ultrasound analysis, to compare calculated stress and strain throughout the left ventricle in stage-29 (ED 6) control hearts and those subjected to pressure overload and underload at stage 21 (ED 3.5). As stress is not a measurable quantity and strain cannot be measured throughout the embryonic ventricle with current techniques, the calculations provide the only current global estimates of stress and strain in these hearts. At stage 21, the muscular intraventricular septum exists only in its primordial form of a more prominent trabecular ridge, the ventricle is filled with a fine network of trabeculae and considerable trabecula-free lumen, and the atrioventricular cushions and endocardial cushions in the common outflow tract are just beginning to mature. By stage 29, the trabeculae have transformed into fenestrated trabecular sheets, compact wall thickness has increased, the intraventricular septum has grown towards the atrioventricular cushions and started to fuse with them, distinct mitral and tricuspid valve primordia develop from the fused atrioventricular cushions, and septation of the outflow tract is almost complete.\textsuperscript{18, 29} Thus this period is crucial in formation of the adult four-chambered structure and myocardial microstructure.

The finite element method has been used in a few studies of the embryonic heart, although to our knowledge this is the first analysis of a trabeculated stage with accurate geometry. Xie and Perucchio\textsuperscript{39} created a voxel-based finite element model of the trabeculated stage-21 chick heart, but modeled the trabeculae locally at the level of small volumes which were then incorporated into a smooth-walled global model. Damon et al.\textsuperscript{5} modeled the stage-21 chick heart as a smooth-walled looped tube by tracing the outer and interior contours from vibratome-sectioned confocal images and smoothing to the innermost trabeculae. Like the current models, it showed higher stresses in the inside of the tube, although not to the degree of the current results.
Measurements of the spatial variation of the stress-strain material relationship at this embryonic stage do not exist; thus, the LV was assumed homogeneous for this study. Note that the stress-strain measurements used for this study are from specimens that include both myocardium and epicardium. The development of more localized and less invasive techniques is needed for more information about the spatial variation of myocardial and epicardial material properties.

Explicit measurements of anisotropy also do not exist, but the assumption of material isotropy is suggested by the embryonic myocardial microstructure, which is randomly organized at this stage. Although Tobita et al.\textsuperscript{34} used confocal scanning with f-actin staining to show that myofiber angles in the stage 27 chick range from -10° to 10°, Wenink et al.\textsuperscript{38} found that even though confocal laser scanning showed myocyte and myofiber orientation in embryonic rat, electron microscopy showed that even by ED 17, myofibrils never completely filled the myocytes and lack of organization was predominant, implying that confocal predictions of myofiber orientation may be misleading. An exception was in ventricular trabeculations, which showed precocious myofiber differentiation. Future experimental measurements may provide more information on the degree of anisotropy in these hearts.

The reference condition for geometry and material properties in this study was the passive, unloaded state, to which mean measured end-diastolic pressure is applied. Flow-induced shear forces are ignored. No measurements of wall shear stress in the stage-29 chick ventricle exist, but these forces are likely significantly lower than the normal pressure. Poelma et al.\textsuperscript{26} calculated wall shear stresses less than 20 Pa in the outflow tract of the stage-17 chick at ejection; these outflow tract stresses should be much greater than those in the left ventricle at end diastole.

As discussed in the Introduction, abundant evidence exists that embryonic myocardial elements sense mechanical loads and respond with load-adaptive growth. The sensing mechanism is unknown. Numerous sensors have been proposed in the mature heart: extracellular matrix,
focal adhesions and integrin-mediated pathways, adherens-junction, titin filaments and z-disk, and cell membrane and cytoskeleton, to name a few.\textsuperscript{13, 17, 25} The actual trigger could be end-diastolic stress or strain, end-systolic stress or strain, peak stress or strain, or some combination of these. This study only considers end-diastolic strain. This choice is based on observations that the passive stress-strain relationship becomes stiffer and LV tissue volume increases after pressure overload. Studies in the mature heart have observed normalization of stress or strain with diastolic pressure\textsuperscript{9, 10, 23}. In the embryonic heart, Lin and Taber\textsuperscript{16} modeled stress-modulated growth in a smooth-walled cylindrical model of the stage 21-29 chick ventricle with end-diastolic pressure as the growth-modulating stimulus and found circumferential growth similar to experimental observations. Tobita et al.\textsuperscript{36} report normalization of LV end-diastolic stress and strain after pressure overload; strain was calculated from epicardial microspheres and stress from pressure based on a thin-walled, uniform-thickness, axisymmetric, ellipsoidal model of the LV.

The calculated mid-ventricular epicardial strains in the models here varied but were in almost all cases less than 10%. Measurements from ultrasound predicted surface strains ranging from 2% to 10%. Although there is general agreement, it is difficult to get point-to-point correlation because of variability in the digital image correlation for strain tracking. The variability is likely a combination of too much out-of-plane motion or torsion, scatter from underlying blood flow, vibration due to probe scanning, and embryonic movement.

Trabecular stresses calculated here are much larger than those in the compact wall. Stress was largest in the smallest trabeculae, at points of connection between trabeculae, and at points of connections between trabeculae and the compact wall. In control models (internal pressure 63 Pa), stresses in the trabeculae were generally larger than 200 Pa, with peaks up to 500 Pa, while stresses at the inner border of the compact wall were generally 100-160 Pa. These inner compact wall stresses agree in magnitude with an analytical estimation for the bounding case of a spheri-
cal smooth-walled shell with linear material properties and no geometrical distortion: the equivalent VM stress based on characteristic lateral dimensions from the control models is 113-138 Pa,\textsuperscript{33} depending on whether the inner or outer trabecular border is used. Models omitting trabecular geometry may therefore not predict true trabecular stress.

Since the model results show a clear spatial variation of stress and strain, if either are a stimulating factor for cell proliferation or changes such as increased myofiber production, a gradient in these effects may occur. Indeed, Jeter and Cameron\textsuperscript{14} showed that although all of the myocardial cells up to stage 32 were in the proliferative pool, proliferative activity is maximal at the periphery of the ventricular myocardium in stage 29-39 chick ventricles, with little proliferative activity in the interventricular septum at stage 29. Grohmann\textsuperscript{11} noted lower proliferation in the muscular interventricular septum formed by trabecular coalescence in the chick. Thus strain, which is largest in the trabeculae, may condition the trabeculae towards differentiation and against proliferation, confirming that biomechanical forces are an important element in ventricular growth and morphogenesis.\textsuperscript{20,30,32,36}

The digital reconstructions verify a significant increase in total tissue volume and cavity volume in hearts overloaded by CTB, agreeing with SEM studies of isolated slices.\textsuperscript{30} This suggests accelerated development, although other markers such as fiber quantity, fiber angles, innervation/excitation patterns, and trabecular patterns were not compared. Based on previous results,\textsuperscript{3} the increased volume is likely due to myocyte hyperplasia.

Although this study showed that changes in mechanical loading over a 2.5-day period influence LV geometry, pressure, and stress-strain relationships, which in turn greatly alter strain in realistic three-dimensional models, strain in the underloaded and overloaded models was not normalized to the control-model strain in a total global sense. In pressure-underloaded models, stresses are lower due to increased volume and decreased pressure, but the stress-strain relation-
ship did not “soften” enough to normalize the volume-strain curves. In contrast, the increased tissue volume and pressure in pressure-overloaded models caused a smaller shift in the volume-stress relationship, but the stiffened stress-strain relationship shifted the volume-strain curve significantly to lower strains. Note that the entire LV volume is weighted equally in these conclusions. Future study of the mechanism of response to altered pressure may suggest refinement of regionalization or globalization criteria.

In conclusion, this study has demonstrated that realistic three-dimensional digital models of the pre-septated, trabeculated left ventricle in control conditions and after altered hemodynamic loading are possible from confocal imaging. Stress and strain throughout the volume can be calculated with realistic loading, material properties, and boundary conditions. Developments in material property characterization can add further refinement, but the current results provide a good starting point. The trabeculae undergo significantly larger stresses and strains than the compact wall; thus, models not including accurate trabecular geometry will underpredict stress and strain. The results could help to explain some features of cardiovascular morphogenesis; perhaps the trabeculae play an important role in sensing and actuating the myocardial response to mechanical load. The calculated values of strain could be used in manipulating cultured cells or more advanced tissue-engineering constructs to obtain desired biological response, such as accelerated differentiation of the contractile phenotype.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Institute of Biomedical Imaging and Bio-engineering, National Institutes of Health (EB002077); National Center for Research Resources, National Institutes of Health (RR16434); Academy of Sciences of the Czech Republic Purkinje Fellowship (D.S.); Ministry of Education, Youth and Sports of the Czech Republic (VZ
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FIGURE CAPTIONS

Figure 1. Image-Processing Flowchart. Raw images from confocal microscopy were imported into the Amira software program. Images were merged in the x-y directions and downsampled to 6-μm cubic voxels. Portions of atria, right ventricle, and valves were removed. Voxel intensities were mapped to pure white and images segmented into tissue and background. Amira generated a tetrahedral surface mesh that was imported into Hyperworks.

Figure 2. A sample volumetric mesh generated by Hyperworks shown by virtual sectioning of a three-dimensional model.

Figure 3. Piola-Kirchhoff stress versus Green’s strain measured from excised tissue strips of stage 29 chick left ventricle. Results for control and pressure-overloaded are from Miller et al.; results from underloaded are new here. Sample sizes were n=19 control, n=18 overloaded, and n=13 underloaded. Data is shown as mean ± S.E. Differences are significant at all strain levels between overloaded and both control and underloaded. Differences are significant between control and underloaded beyond 10% strain.

Figure 4. Color contour plots of von Mises stress calculated with LS-DYNA for a characteristic model in each of the three groups. Bar scale shows stress in Pa. Scaling is not necessarily equivalent on all models and cutting planes were chosen to show maximal interior view.
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Figure 5. Histogram of percentage of left ventricular tissue volume versus von Mises stress. For clarity, results are shown as bin height plotted versus the bin center and points are connected. Values are mean ± SE of all models in the group. * denotes percentage volume of the overloaded or underloaded models is significantly different from the control group at the specific stress bin (p<0.05). + denotes percentage volume of overloaded models is significantly different from underloaded models (p<0.05).

Figure 6. Color contour plots of four different stress measures for a single heart in the control group. Bar scale at left denotes stress in Pa for cases (a), (b), and (c). Bar scale in lower right denotes stress in Pa for case (d).

Figure 7. Color contour plots of von Mises strain calculated with LS-DYNA for a characteristic model in each of the three groups. Bar scale shows strain (non-dimensional). Scaling is not necessarily equivalent on all models and cutting planes were chosen to show maximal interior view.

Figure 8. Histogram of percentage of left ventricular tissue volume versus von Mises strain. For clarity, results are shown as bin height plotted versus the bin center and points are connected. Values are mean ± SE of all models in the group. * denotes percentage volume of the overloaded or underloaded models is significantly different from the control group at the specific strain bin (p<0.05). + denotes percentage volume of overloaded models is significantly different from that of underloaded models at the specified strain bin (p<0.05).
(a) Control  (b) Pressure-Underloaded  (c) Pressure-Overloaded
Figure 6

Common Links: Click Here To Download High Resolution Image

(a) 1st principal stress

(b) 2nd principal stress

(c) 3rd principal stress

(d) Von Mises stress
Table 1. Parameters for best fit of Ogden material model to experimental stress-strain measurements.

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<td>-0.4480E+01</td>
</tr>
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<td>-0.2734E-07</td>
<td>0.3199E+00</td>
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<td>0.1270E-08</td>
<td>0.7904E+01</td>
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<tr>
<td>Overloaded</td>
<td>-0.4925E-10</td>
<td>-0.8480E+01</td>
</tr>
<tr>
<td></td>
<td>-0.3521E-06</td>
<td>-0.1610E-01</td>
</tr>
<tr>
<td></td>
<td>-0.2991E-08</td>
<td>0.3984E+01</td>
</tr>
<tr>
<td></td>
<td>0.1213E-08</td>
<td>0.7984E+01</td>
</tr>
</tbody>
</table>
Table 2. Left ventricular tissue and cavity volumes for the reconstructed confocal images of the three treatment groups.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Left Ventricular Tissue Volume (mm$^3$)</th>
<th>Left Ventricular Cavity Volume (mm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.88 ± 0.05</td>
<td>0.61 ± 0.06</td>
</tr>
<tr>
<td>Underloaded</td>
<td>1.00 ± 0.11</td>
<td>0.55 ± 0.09</td>
</tr>
<tr>
<td>Overloaded</td>
<td>1.33 ± 0.08*</td>
<td>0.91 ± 0.10*</td>
</tr>
</tbody>
</table>

* p<0.01 vs control; †p<0.05 vs control and underloaded
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