Residual Strain in Rat Left Ventricle

Jeffrey H. Omens and Yuan-Cheng Fung

Residual stress in an organ is defined as the stress that remains when all external loads are removed. Residual stress has generally been ignored in published papers on left ventricular wall stress. To take residual stress into account in the analysis of stress distributions in a beating heart, one must first measure the residual strain in the no-load state of the heart. Residual strains in equatorial cross-sectional rings (2–3 mm thick) of five potassium-arrested rat left ventricles were measured. The effects of friction and external loading were reduced by submerging the specimen in fluid, and a hypothermic, hyperkalemic arresting solution containing nifedipine and EGTA was used to delay the onset of ischemic contracture. Stainless steel microspheres (60–100 μm) were lightly imbedded on the surface of the slices, and the coordinates of the microspheres were digitized from photographs taken before and after a radial cut was made through the left ventricular free wall. Two-dimensional strains computed from the deformation of a slice after one radial cut were defined as the residual strains in that slice. It was found that the distributions of the principal residual stretch ratios were asymmetric with respect to the radial cut: in areas where substantial transmural strain gradients existed, the distributions of strain components were different on the two sides of the radial cut. A second radial cut produced deformations significantly smaller than those produced from the first radial cut. Hence, a slice with one radial cut may be considered stress free. Our results show that the circumferential residual strain was negative in the endocardial region (stretch ratios were significantly less than 1.00, p<0.001 for five slices), while those in the epicardial region were either positive or negative with a much smaller magnitude. This residual strain distribution suggests that, in general, there is compressive circumferential residual stress at the inner layers of the ventricle. A residual stress distribution of this nature may reduce the endocardial peak in circumferential tensile stress at end diastole predicted by analytical models using nonlinear material properties. (Circulation Research 1990;66:37–45)

A

lysis and use of residual stress, defined as the stress in a body when all external loads are removed, have been part of mechanical engineering for well over a century. Only in the past few years have these ideas been applied to biological tissues. Several recent analyses of the arteries have taken the initial stress into account, resulting in decreased transmural circumferential stress gradients when the vessel is inflated. Without residual stress, tensile circumferential stress at physiological arterial pressures can be up to 10 times as large at the inner wall compared with the outer wall.

An accurate biomechanical analysis of stress and strain must use the zero-stress state as a reference state. Once the stress-free state is defined, residual stress and externally loaded stress states may be analyzed. Assuming that residual stress exists, the first step in a stress analysis is the quantification of residual strain, or the strain when a body is deformed from the stress-free state to the no-load state. Once the residual strain is known, then an appropriate constitutive law, referred to the stress-free state, can be employed to calculate the stress. The aim of the present study is to quantitatively describe the two-dimensional residual strains in an equatorial slice of the rat left ventricle.

At the present time, no experimental measurement of residual strain in the myocardium exists. Most stress analyses assume the unloaded left ventricle to be stress free. Critical reviews of some of these models have been published by Huisman et al, Yin,8 and Pelle et al.9 Many of these mathematical models show large stress concentrations at the endocardium when the ventricle is passively inflated. This effect is more pronounced when nonlinear constitutive properties are used.7,10 If a compressive residual stress were present at the inner layers of the ventricular wall, then end-diastolic endocardial stress
concentrations will be reduced. Hence, the end-diastolic circumferential stress may be more uniform across the left ventricular wall thickness.

Our initial experiments on left ventricular residual stress used whole hearts with a meridional slit entirely through the left ventricular wall.\textsuperscript{11,12} These initial experiments showed the existence of residual stress, as well as a distinctive asymmetrical deformation pattern of the left ventricle after the slit was cut. In the mean time, an arresting protocol and cardioplegic solution were developed, which deterred the effects of ischemia while preserving the diastolic mechanical properties of the tissue. To more accurately quantitate the residual strain, latitudinal slices of the heart were made first, followed by a radial cut, whereby the surface strain could be directly measured. These strains are only a small sampling of the many residual strain components that are present in the left ventricle. Even with the limited results for residual strain obtained so far, general characteristics of residual stress may be surmised.

It is known that biological tissues can adapt to external stress and strain. Bone remodeling is an example that has been well documented.\textsuperscript{13} It is thought that cardiac hypertrophy and growth develop in response to changes in diastolic and systolic wall stress.\textsuperscript{14} Our general hypothesis is that growth and change affect the stress and strain in a tissue, more specifically the residual stress and strain. The zero-stress state of an organ displays the natural shapes and sizes of the cellular and extracellular structures of the organ in the undeformed condition. If the cells or extracellular matrix remodel by hypertrophy, hyperplasia, or resorption, it is necessary to describe the change in shape and size of the cells and matrix; a simple way to do this is to describe the change of the zero-stress state. Hence, the changes in zero-stress state, or equivalently the residual stress and strain, reflect tissue remodeling.

Materials and Methods

Isolated Heart Preparation

Five adult male (200–300 g) Sprague-Dawley rats were anesthetized with pentobarbital (50 mg/kg) intraperitoneally. A tracheostomy was performed, and the animal was ventilated with 70% O\textsubscript{2}-30% N\textsubscript{2} gas. After 10 minutes of ventilation, the heart was arrested by clamping the ascending aorta and injecting an icied, heparinized (10,000 units/l) hyperkalemic Krebs-Henseleit solution directly into the left ventricle through the apex. The perfusate contained (in g/l) NaCl 5.40, Na\textsubscript{2}SO\textsubscript{4} 0.22, Na\textsubscript{2}H\textsubscript{4}PO\textsubscript{4} 2.08, KCl 1.87, MgCl\textsubscript{2} • 6H\textsubscript{2}O 0.23, and dextrose 1.0 and was bubbled with 95% O\textsubscript{2}-5% CO\textsubscript{2} gas. Also added to the perfusate were the calcium channel blocker nifedipine (2×10\textsuperscript{-4} g/l) and the calcium chelator EGTA (10 mM/l). The arrested hearts were rapidly removed, and the ascending aorta was cannulated for coronary perfusion at 20\textdegree–23\textdegree C for 2–3 minutes using the hyperkalemic solution with approximately 50 mm Hg pressure.

Equatorial Slice and Radial Cut

In defining the residual strain, the stress-free state was used as the reference state. Since cutting the tissue causes the surface traction\textsuperscript{*} acting on the cut surface to vanish, the stress-free state can be revealed by cutting the tissue in enough places to relieve all of the residual stress components. If a thin slice of tissue is obtained from the ventricle, then the stress components perpendicular and tangential to the cut surface of the slice become zero, and the state of stress in the thin slice is that of the so-called "generalized plane-stress" state, which is two-dimensional.

Slicing of the rat heart was a simple and fast procedure. The arrested, isolated heart was secured with a pin at the apex. Holding the aortic cannula at the other end, a single-edged razor blade was used to slice the heart perpendicular to its axis in the equatorial region (Figure 1A). The slices were 2–3 mm thick and were always analyzed with the base end facing up.

A preliminary study was performed using epicardial markers that showed that this slicing technique did not produce any measurable deformation in the circumferential direction. In other words, within the experimental accuracy, the shape of the equatorial slice was the same as its configuration while part of the intact ventricle.

After slightly drying the surface of the slice with an absorbent cloth, stainless steel microspheres (60–100 \textmu m diameter) were sprinkled on the slice and lightly pressed into the tissue. The illuminated microspheres served as a grid for strain field measurements. With the microspheres in place, the slice was moved to a small dish containing the same perfusate as previously described at room temperature. The slice was completely submerged in the fluid, and any loose microspheres were rinsed from the surface. The slice assumed its natural shape resting on a plastic pedestal. The surrounding fluid counteracted the gravita-

\textsuperscript{*}Surface traction, or stress vector, represents the force per unit area acting on the surface.
tional and frictional forces, leaving the slice in the no-load state. After a photograph was taken, the free wall of the left ventricle was cut radially (Figure 1B) at a location opposite to the right ventricle and photographed within 30 seconds of radial cutting. It was imperative that the photograph of the radially cut configuration be taken as quickly as possible so that extraneous deformations resulting from contraction were not recorded. Photographs of an equatorial slice before and after radial cutting are shown in Figure 2. After the radial cut was made, the slice was considered to be in the “stress-free” state.

**Opening Angle**

A simple quantitative measure that reflects the residual strain distribution in a slice of the left ventricle is the opening angle (Figure 3C). Although the opening angle cannot give the same description of strain as the deformation analysis described in the next section, opening angles may be incorporated into analytic and continuum mechanics models for stress and strain. The opening angle of a thin latitudinal slice of the left ventricle was defined as the angle between the two radial lines connecting the center of the ventricular chamber and the centerlines of the walls at the cut edges. The steps used to estimate the chamber center and opening angle in the stress-free state are outlined in Figures 3A and 3B.

**Strain Calculation**

The basis for our finite strain distributions is the homogeneous strain analysis from two-dimensional continuum mechanics theory. This method has been previously used for calculation of myocardial two-dimensional finite strains using ultrasonic crystals, and has been extended for measuring myocardial three-dimensional finite strains using radiopaque myocardial implants. The basic assumption of this analysis is that the strain in an area defined by three planar points is homogeneous (constant). Selected microsphere positions were digitized in the no-load and stress-free states from the enlarged photographs. The position of each microsphere was digitized five times and averaged. The x-y coordinate data were used to estimate the strain components in each area defined by three selected points forming a “trial.” The finite Lagrangian strain components were calculated from

\[
\Delta \varepsilon^2 - \Delta \varepsilon_0^2 = \sum_{i=1}^{2} \sum_{j=1}^{2} 2E_i \Delta a_i \Delta a_j,
\]

where \(\Delta \varepsilon\) and \(\Delta \varepsilon_0\) are the lengths of each side of the triad in the stress-free and no-load states, respectively, and \(\Delta a_i\) and \(\Delta a_j\) are the respective x and y components of \(\Delta \varepsilon_0\). To calculate the three independent strain components \(E_i\), three linear equations were written for the three sides of the triad, and solved simultaneously.

The values of principal strains are of interest. By solving an algebraic eigenvalue problem, the principal strains \(E_1\), \(E_2\) and the directions of the mutually orthogonal principal axes were readily calculated. The principal stretch ratios were then calculated using

\[
\lambda_i = (2E_i + 1)^{1/2}, \quad i = 1, 2.
\]

The principal stretch ratios, \(\lambda_1\) and \(\lambda_2\), were determined from principal strains, \(E_1\) and \(E_2\), and represent the ratios of the segmental length at zero stress to that at no load. The principal stretch ratios are the maximum and minimum of the stretch ratios of line elements in all directions initiated from a single point. In the principal directions there is no shear.

Note that from the equations given above, the principal directions are referred to the no-load state. When describing a stretch ratio or a strain, one must state to which configuration the value refers to. Since
our definition of residual strain is referred to the stress-free state, each principal stretch ratio presented in the following sections is simply the inverse of the stretch ratio found from the previous equations.

Results

The present investigation is concerned with the residual strains in equatorial slices of arrested myo-
To examine statistical differences in these strain distributions, we have combined data from five equatorial slices. The transmural stretch ratios have been divided into three regions, each comprising 33% of the wall thickness, and are referred to as endocardial, midwall, and epicardial. In all cases, the wall of the right ventricle and the left ventricular papillary muscles have been ignored. In the no-load configuration, each slice has been further divided into four 90° quadrants, with the position of the cut used as a reference (see Figure 5). Near the center of each quadrant, a representative stretch ratio distribution has been found, and the two principal stretch ratios have been averaged for each of the three transmural regions. Thus, each slice gives 12 average stretch ratios in each of the principal directions. Figure 7 shows the means and standard deviations from five such slices.

The average endocardial circumferential stretch ratio from all four quadrants was 0.911 ± 0.055 (mean ± 1 SD). A Student’s t test shows this to be significantly less than $\lambda = 1.00$ ($p < 0.001$). Thus a significant compressive endocardial prestrain in the circumferential direction exists in each of the four quadrants. The epicardial circumferential stretch ratios were greater than 1.00 in quadrants 1 and 2 ($p = 0.006$ and 0.012, respectively), with no significant differences in quadrants 3 and 4. Therefore, a significant difference exists between endocardial and epicardial circumferential stretch ratios. The stretch ratio distributions in quadrants 1 and 4 have different patterns. A t test comparing groups from quadrants 1 and 4 shows significant differences in both the circumferential ($p = 0.004$) and radial ($p = 0.011$) epicardial stretch ratios, with no significant differences in the endocardial or midwall values. Thus, there is evidence for posterior wall versus anterior wall strain distribution variation.

**Additional Deformation due to an Additional Cut**

To answer the question of whether one cut is enough to relieve all residual stress in a slice of myocardium, a few experiments were performed in which a second radial cut was made directly opposite to the first. This second cut relieves normal and shear stresses remaining in the specimen along the second cut surfaces. The second cut induces additional deformations. These additional strains were measured referenced to the single cut configuration (given in terms of principal stretch ratios). Figure 8 shows the stretch ratios at one circumferential location (shown below plots) after one, and then after a second radial cut. The stretch ratios due to the second cut are referred to the configuration after the first radial cut. The mean stretch ratios along the chosen radius resulting from the second cut are 0.990 ± 0.007 and 1.019 ± 0.018 (mean ± 1 SD) for $\lambda_1$ and $\lambda_2$, respectively. The differences of these numbers from unity are small enough that they lie within the limits of experimental accuracy of the method. Hence, the additional strain due to the second cut can be considered small when...
compared with that after one cut, and a slice with one radial cut is considered to be free of stress.

**Discussion**

When a radial cut is made through an equatorial slice of the left ventricle, the ring opens up to an arc, showing the existence of residual stress. Although it may be possible to measure these residual stresses directly using some strain gauge devices, an accurate method to measure the myocardial forces directly is unknown. At the present time, the only way to accurately assess the residual stresses in the myocardium is to calculate them from analytical or finite element models once the residual strains have been measured. Even if the experimental measurements are precise, the calculation can only be as accurate as the assumed stress-strain relation.

For diastolic residual strain measurements, ideally the diastolic mechanical properties of the myocardium should be preserved. Although the mechanical properties of the potassium-arrested myocardium are probably different from the diastolic properties of contracting muscle, our experimental protocol was designed to measure strain in arrested myocardium without contracture. Ischemic contracture will alter the mechanical properties of the tissue and hence change the measured residual strains. Certain interventions can reduce ischemic contracture, such as hyperkalemic arrest and hypothermia. A calcium channel blocker may provide increased protection.
against contracture during cardioplegia. Another perfusate additive that may help prevent contracture is the calcium chelator EGTA. Holubarsch et al. used a calcium-free Tyrode’s solution with 10 mM EGTA in isolated papillary experiments, and showed that the active tension was completely abolished without changing initial resting tensions. These perfusate additives should help the arrested myocardial tissue retain its diastolic mechanical properties. The gradual increase in observed opening angle is presumably due to the effects of ischemic contracture. Our results have shown that arresting the heart with a hypothermic, hyperkalemic solution with EGTA and nifedipine delayed the adverse mechanical effects of contracture, since the opening angle remained constant for the first few minutes after radial cutting.

We have chosen to present the principal strains (stretch ratios); hence, normal and shear components of the strain tensor are not explicitly given. If the principal angles correspond to the local circumferential and radial directions, the shear strains will be zero, and the radial and circumferential components will be equal to the two principal strains. For simplicity, the radial direction is assumed to always pass through the center of the no-load ventricular slices. Due to the irregular geometry of the slices, the estimated local radial and circumferential directions may not be accurate. Even though our calculated principal directions were statistically different from the radial and circumferential directions found directly from the slice geometry, the example of Figure 6 shows an average absolute difference of about 14°. As an example, if the principal strains are 0.1 and −0.1 (λ = 1.095 and 0.905), and the principal angle is 14°, the in-plane shear strain is 0.024. Other slices show similar values for principal directions. The technique does not account for rigid body motions or rotations. These quantities do not contribute to the strain tensor and must be considered separately.

The measured opening angle depends on the location chosen for the center of the ventricle. If the center is moved 1 mm (on the scale of the slice) in the anterior-posterior direction, then the opening angle changes by approximately 2°. If the center is moved 1 mm perpendicular to the anterior-posterior direction, the opening angle will change by about 7°. Since

Figure 7. Bar graph of principal stretch ratios represented as mean and standard deviation for five equatorial slices. The slices have been divided transmurally into three equal regions (Endocardial, Midwall, and Epicardial) and circumferentially divided into four quadrants (Q1–Q4). The quadrants are shown in Figure 5. Near the center of each quadrant, the transmural distributions of the two principal stretch ratios were found and the data were averaged for each of the three transmural regions. λ1 is referred to as the “circumferential” principal stretch ratio, and λ2 is the “radial.”

Figure 8. Principal stretch ratios after one radial cut, and the additional deformation due to a second radial cut. These distributions are taken from an area near the first cut (shown below plots). The stretch ratios after a second cut are referred to the configuration after the first cut. Within the experimental accuracy, the second cut does not result in substantial deformations compared with those after the first cut.
the inner diameter of a typical slice is <5 mm, it is unlikely that the estimated center would differ by more than 1 mm in two similarly shaped slices. Variability in principal strains may arise from several sources. The resolution of the photographs is extremely high. Errors arise when microsphere positions are digitized. Typically, distances measured from an enlarged photograph are on the order of 1 cm. The digitization error for this length is ±0.02 cm. Using the method described by Kline and McClintock, the uncertainty in the calculated stretch ratios becomes 2.8%.

The location of the radial cut may affect the measured residual strains, as well as the opening angle. The location of the equatorial slice may also influence the strain distributions. Preliminary results from slices in different regions (closer to the base or apex) have shown different residual strain patterns. Other sources of variability include ischemic contracture in the tissue, frictional interaction between the specimen and the material on which it rests, and possible movement of the microspheres, especially when the radial cut is made.

The residual strains of equatorial slices measured in this study do not conform to a simple, regular pattern. Slices from similar locations in different hearts do show somewhat predictable strain patterns. The anterior free ventricular wall (right side of slices) has a strain distribution close to what might be expected from pure bending of the ventricular wall: a transmural gradient of circumferential principal stretch ratio with endocardial values less than unity and epicardial values greater than unity. Near the intraventricular septum, the maximum stretch ratios are smaller in magnitude. On the posterior ventricular free wall (left side of slices), the strain pattern does not suggest simple bending of the wall. In all cases, the endocardial circumferential stretch ratios are less than unity. This trend is significant for all of the locations around the circumference. In general, this points to a compressive residual circumferential stress for this portion of the left ventricular wall. This is an important feature when considering stress concentration reduction at the endocardium. The non-axissymmetric distribution of residual strain may be due to regional variations in wall thickness and wall curvature, or possibly caused by mechanical interaction with the papillary muscles.

Residual strain is defined with respect to the zero-stress state. The tissue is completely free of stress only if an arbitrary cut does not cause a deformation. In our myocardial slices, the stretch ratios resulting from a second radial cut are small compared with those obtained after the first cut, particularly in regions further removed from the second cut. This is expected from the well-known St. Venant principle because the resultant normal and shear forces and bending moment must vanish over the second cut. The St. Venant principle states that in an elastic body, the influence of any system of loads with zero resultant forces and moments will vanish as the distance from the loads increases indefinitely. In all probability, however, local residual stress still exists in the slice even after two radial cuts have been made. The only way to relieve all of these local residual forces may be with innumerable "microscopic" cuts. At the present time, this is not experimentally feasible. Using a continuum approach, these local stress "concentrations" are considered to exist on a much smaller scale than the dimensions of the slice. From this point of view, we may assume that the stress-free state is closely represented by an equatorial slice with a single radial cut.

The opening of the radial cut may be influenced by other factors besides the residual stress such as localized tissue damage near the cut surfaces, interstitial fluid movement and changes in interstitial pressure, and ischemic contracture. Even if our preparations are free from such effects, the components of the ventricular wall that "create" the residual stress have not been identified. Likely candidates include the myocytes and/or the collagen network. Studying residual stress and strain in the hypertrophic heart, wherein the collagen and muscle cells may change in size, shape, or rheological properties, may help resolve the question as to which components are important in the generation of the residual forces. Residual stress has many possible implications to the physiology of the left ventricle. Direct implications may include altering the diastolic stress distribution in the ventricle in such a way as to lower the endocardial tensile stress components, changes in hypertrophic adaptation, and alteration of myocardial blood flow patterns. Indirectly, residual stress can affect dynamic features of the heart such as force of contraction, stroke volume, and tissue oxygen consumption by changing the end-diastolic stress distribution, that is, the preload on each muscle fiber. It is evident that residual stress may be an important factor in many facets of cardiac dynamics.

The present description of residual strain in the left ventricle is far from complete. Three-dimensional residual strain fields must be found for the entire left ventricle. Although the present method cannot measure strains perpendicular to the plane of the slice, in all probability residual stresses and strains exist in this direction and should be quantified before a three-dimensional model for residual stress is formulated. Even though an accurate model for residual stress in the left ventricle has not been developed, the zero-stress/zero-strain reference state should be carefully considered in analyses of ventricular wall stress and ventricular mechanics. A valid model predicting left ventricular residual stress should include the large deformations of a nonlinear elastic material anisotropic with respect to a fiber axis changing continuously through the three-dimensional geometry. Many cardiologists, using relatively simple linear models for stress, often wrongly assume that wall stress is zero when the ventricular pressure is zero.

In conclusion, two-dimensional residual strain distributions have been found for equatorial slices of
potassium-arrested rat hearts. Residual stresses are relieved in a thin slice with a single radial cut opposite to the right ventricle. A second radial cut resulted in small deformations, indicating that a thin slice with a single radial cut is essentially stress free. The slices consistently opened up to an arc with an opening angle of about 45°. The residual principal strain distributions corresponding to this opening were calculated and showed significant differences on the posterior and anterior walls. In all cases, the endocardial circumferential principal strains were negative, which suggests a compressive residual circumferential stress component. A residual stress distribution of this nature may reduce tensile endocardial stress concentrations predicted by mathematical models of ventricular wall stress using nonlinear constitutive material properties.

Acknowledgment

We wish to acknowledge Dr. Andrew D. McCulloch for his helpful discussions and review of this manuscript.

References

11. Omens JH: Left ventricular strain in the no-load state due to the existence of residual stress (doctoral thesis). University of California San Diego, La Jolla, Calif, 1988

KEY WORDS • residual stress • initial stress • initial strain • zero-stress state