Changes in Passive Mechanical Stiffness of Myocardial Tissue With Aneurysm Formation

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Background  Myocardium undergoes complex cellular and histochemical alterations after acute myocardial infarction. These structural changes directly affect the mechanical stiffness of infarcted and remote myocardia. Previous investigations of infarct stiffness have been limited to uniaxial testing, which does not provide a unique description of the tissue's three-dimensional material properties. This study describes the first serial measurements of biaxial mechanical properties of sheep myocardium after anteroapical infarction.

Methods and Results  Anteroapical infarctions of 23.7±2.5% of the left ventricular mass were produced by coronary arterial ligation in sheep. Biaxial force-extension measurements were made on freshly excised squares (6.45 cm²) of remote, noninfarcted, and infarcted myocardia before and 4 hours, 1 week, 2 weeks, and 6 weeks after ligation. Adjacent myocardial samples were assayed for hydroxyproline content. Force-extension data and a derived constitutive equation were used to describe stresses and strains and material properties of each sample. In sheep, anteroapical infarctions evolve into thin left ventricular aneurysms that consist of predominantly fibrous tissue with disrupted groups of muscle cells encased in scar. In the infarct, Cauchy stresses at 15% extensions (control stresses: circumferential, σc, 19.4±3.3 g/cm²; longitudinal, σl, 54.8±34.8 g/cm²) increase within 4 hours, peak at 1 to 2 weeks (σc, 338.5±143.6 g/cm²; σl, 310.7±45.9 g/cm²), and then decrease 6 weeks after infarction (σc, 115±47.2 g/cm²; σl, 53.2±28.9 g/cm²). Stresses in the remote myocardium follow a similar time course but to a lesser extent than the infarcted region. Hydroxyproline content, a measure of collagen content, does not correlate with infarct stiffness but progressively increases to 69.7±7.6 µg/mg after 6 weeks. Stress-extension curves demonstrate directional anisotropy of both infarcted and remote myocardia.

Conclusions  The findings indicate that infarcted myocardium becomes more stiff during the first 1 to 2 weeks after anteroapical infarction and then more compliant. The infarct also exhibits directional anisotropy. These observations underscore the importance of ventricular material properties during the remodeling process after acute myocardial infarction and may partially explain the progressive left ventricular dilatation and functional deterioration that occur in some patients after anteroapical infarction. (Circulation. 1994;89:2315-2326.)

Key Words  • stiffness • myocardium • infarction • aneurysm

Acute myocardial infarction causes complex structural alterations that eventually lead to collagen synthesis and scar formation. Early after infarction, edema and infiltration of inflammatory cells1,2; loss of collagen matrix that extends beyond necrotic muscle cells3; infarct expansion and thinning2,4; and histochemical events leading to production of intra- and extracellular collagen1,5-6 occur. By the first week, there is deposition of immature collagen, increase in fibrillablast formation, and increased resorption of necrotic cells.1,6 By the second week, there is total loss of normal collagen matrix, followed by abnormal replacement with mature collagen.3,6 Viable borderzone myocardium that previously lost collagen struts is now encased in a fine collagenous membrane.7 Over time, there is continued resorption of necrotic tissue and replacement with scar tissue and some viable muscle cells. There also is a tendency for fibril thickening and collagen bundle alignment along stress lines.3 These complex cellular and histochemical changes directly affect the mechanical stiffness of myocardium, which can be described by its passive material properties.

Material properties of infarcted myocardium are an important determinant of global pump function and the contractile effectiveness of noninfarcted myocardium. Nonetheless, material properties also may occur in regions that are not directly infarcted: altered stresses in the infarcted ventricle may induce remodeling that leads to stiffness changes in other regions of the ventricle. Several investigators have directly measured infarct stiffness.5,8-10 However, these measurements have all been made under uniaxial loading and therefore do not provide a unique description of three-dimensional material properties. This point has been made by Yin11 Abe et al,12 and Moriarty,13 who explain that two materials with significantly different behavior under biaxial loading can have identical uniaxial stress-strain relations. Because biological tissue is generally considered incompressible,14 biaxial tissue testing is preferable for describing three-dimensional constitutive relations.15 To date, there is no detailed study of infarct properties using a direct, biaxial testing procedure.

Mechanical properties of myocardium, as well as for most soft tissue, are known to be inelastic, ie, there is a multivalued relation between stress and strain. Thus,
Fung has coined the term "pseudoeclasticity" to describe finite elastic theory used in characterizing tissue material properties. For the purposes of comparing noninfarcted and infarcted myocardia, it is convenient to define constitutive equations based on finite elasticity. The material parameters from these equations can be directly used to quantify mechanical changes due to infarction.

We developed biaxial tissue tests used to determine stiffness (elasticity) of both infarcted and noninfarcted myocardia. We used an experimental model of an anterioapical myocardial infarction in sheep that produced large, reproducible, transmural infarcts that uniformly progressed to ventricular aneurysm similar to that seen in humans. Infarcts of varying ages were excised and subjected to direct mechanical testing using a biaxial tissue-stretching apparatus. Statistical comparison of myocardial stiffness with infarct age was performed based on material properties computed from mathematically modeling the stress-strain curves using a constitutive relation based on the theory of finite deformation elasticity.

**Methods**

A total of 29 adult Dorsett sheep (38.6±6.8 kg) were studied. All animals were studied in compliance with National Institutes of Health publication No. 85-23 as revised in 1985. Biaxial mechanical tests were performed on sections of the left ventricular free wall from normal ovine hearts and noninfarcted and infarcted sections from hearts 4 hours to 64 days after induction of myocardial infarction.

**Creating the Left Ventricular Aneurysm**

Anterioapical infarcts were created in 23 ovine hearts using the procedure previously developed by our group. Anesthesia was induced with sodium thiopental (25 mg/kg IV) and maintained with isoflurane (2.0% to 3.0%) after intubation and mechanical ventilation (tidal volume, 15 mL/kg; model AVEC, North American Drager). Glycopyrrolate (0.2 mg IV) was given at induction to control bronchial secretions. The chest was entered through a sterile left thoracotomy. An anterioapical infarct was produced by ligation of the left anterior descending and second diagonal coronary arteries at a point 40% of the distance from the apex to the base. All infarcted animals were transferred to a farm while the infarct evolved into an anterioapical aneurysm.

**Harvesting the Tissue Samples**

Tissue samples were harvested from hearts at various times after infarction. Six hearts were studied from animals without myocardial infarction; these represent the control group. Three hearts were studied 4 hours after ligation of coronary arteries; these were designated the 4-hour group. Eight hearts were studied from animals with infarcts ranging from 6 to 9 days; these constituted the 1-week group. Five hearts were studied from animals with infarcts from 13 to 14 days; these constituted the 2-week group. Finally, seven hearts were studied from animals with infarcts from 39 to 62 days; these constituted the 6-week group.

Sheep were killed, and hearts were excised at the above intervals after infarction. Anesthesia was induced as described above. Through a clean left thoracotomy or median sternotomy, the heart was exposed. Potassium chloride (saturated, 120 mL) was injected into the left ventricular cavity to produce immediate asystolic arrest. The heart was immediately excised, placed into cold (0 to 5°C) isotonic cardioplegic solution (14 mmol/L KH₂PO₄, 11 mmol/L KOH, 15 mmol/L KCl, 220 mmol/L glucose, 0.1 mmol/L EGTA, 75 μmol/L adenosine; pH 7.4), and rapidly transported to the biaxial tissue-stretching apparatus for study.

**Biaxial Tissue-Stretching Apparatus**

The biaxial tissue-stretching system (Fig 1) consists of five parts: (1) a microcomputer-based control system, (2) two force transducers, (3) custom-designed tissue clamps that allow biaxial motion without tissue distortion, (4) an optical system for measuring tissue displacement, and (5) a physiological water bath. As shown, the tissue sample is placed into a bath, and all four edges are held by clamps.

In each axis, one movable clamp is attached to a stepping motor-driven linear actuator. The opposing clamp is stationary and attached to a force transducer. The stepping motors operate under the control of a microcomputer. The clamps to
hold the sample are designed to allow biaxial stretching without tissue distortion. Briefly, the tissue is held along each edge by an array of pins that pass perpendicularly through the tissue. Each pin (0.7-mm-diameter stainless steel wire) also passes through a pair of hourglass-shaped stainless steel rollers (3.18-mm maximal outer diameter; 1.59-mm minimal outer diameter; 0.79-mm inner-bore diameter; 7.14 mm high) situated above and below the specimen. The rollers, in turn, ride along tracks formed by pairs of parallel stainless steel rods (2.36-mm diameter; 4.37-mm center-to-center spacing). Thus, there is a pair of tracks along each of the tissue edges. The net effect of the roller clamps is to allow rectangular samples to remain rectangular under large deformations. Complete tissue clamping and mounting require less than 5 minutes.

Central strain was measured from surface markers. Specifically, two pairs of brightly colored, thin stainless steel pins (0.610-mm outside diameter) were placed through the tissue along the two orthogonal axes of the stretcher. Each pair of pins was situated in the central region of the sample along one of the axes of the stretcher. The pin displacements were recorded using a video camera (WV-5260/8AF video camera, 84-mm macro lens, 1/1000-second high-speed shutter, Panasonic) mounted above the stretcher bath.

While they were in the biaxial stretching apparatus, tissue samples were immersed in a thermally regulated (20°C) isotonic cardioplegic solution (14 mmol/L KH₂PO₄, 11 mmol/L KOH, 15 mmol/L KCl, 220 mmol/L glucose, 0.1 mmol/L EGTA, 75 μmol/L adenosine, pH 7.4).

Experimental Protocol

After transport to the biaxial tissue-stretching apparatus, separate transmural 2.54×2.54-cm tissue samples were harvested from the remote, noninfarcted tissue at the left ventricular anterior base and from clearly infarcted tissue at the anterior apex. Samples were maintained in cold (0 to 5°C) cardioplegic solution during preparation. Each sample was marked with a suture to indicate orientation. Thicknesses of each sample were measured at five locations with a custom-built electrical resistance micrometer, and sample thickness was defined as the mean of the five thickness measurements.

The tissue samples were mounted on the biaxial stretcher. The alignment was always such that one orthogonal stretching axis was aligned in the circumferential direction of the tissue sample. Before any data collection, a reference length for the sample was determined. The reference length was defined as the length at zero force. This length was measured while applying small, cyclic, equibiaxial ramp displacements (typically 6% to 10% of the tissue length) to the sample. During this cyclic loading, outputs from both force transducers were monitored. As the clamps cycled back and forth, small adjustments in the clamp-to-clamp separations were made until the minimal loads seen on both axes were near zero. Care was taken to avoid overstressing the sample to prevent load-dependent material stiffness changes.17,18 In practice, it is not always possible to achieve zero-load levels simultaneously along both axes. In these situations, the reference lengths were said to be found when the minimal loads on both axes were within ± 1 g. The stretching was then interrupted, and the reference lengths for each axis were measured with an internal caliper (model DCG, 1.4-in., MTI, Mitutoyo). The reference length was taken as the separation between the array of pins passing through one tissue edge and the array of pins passing through the opposite edge.

After the reference lengths were determined, each sample underwent a series of cyclic equibiaxial stretches. During the first stretching cycle, the displacements applied to the sample were on the order of 6% to 10% of the reference lengths. With each successive stretching cycle, the applied displacements were serially increased until maximal stretches of approximately 20% to 25% were reached. Some infarct samples were too inextensible for the force transducers; in such cases, stretching extensions were increased only up to the maximal measurable loads. The stretching rate for all cyclic runs was 0.5 Hz. Each data run was preceded by 10 preconditioning cycles.

A typical stretching protocol consisted of nine equibiaxial stretches in a set order: 8.5%, 10.0%, 11.5%, 12.9%, 14.5%, 16.1%, 17.5%, 19.1%, and 20.6%.

Data Collection and Analysis

For each run, the sample deformation was recorded on a digital video recorder (AG-1950 videocassette recorder, Panasonic), and the two forces and two clamp-displacement signals were digitally sampled by a 16-channel, 12-bit analog-to-digital converter (Lab Master DMA, Tecmar Inc) housed in an IBM AT-class personal computer. All signals were sampled for 10 seconds at 100 Hz and later filtered with a 20-Hz low-pass digital filter.

Stress-strain curves were constructed for each data run. Stress and strain calculations were made with reference to Fig 2, which shows a schematic of a deformed rectangular tissue sample. Because the stretching axes were aligned with the circumferential and longitudinal directions of the tissue sample, we use the subscript C to denote quantities related to the circumferential stretching direction and the subscript L to denote the longitudinal stretching direction. The clamp design of the biaxial stretcher minimizes shear stresses acting on the tissue edges with the equibiaxial stretching protocol. Therefore, the stretching directions are the principal directions. As shown in Fig 2, Fc, represents the forces imposed on the sample in the ith stretching direction (circumferential or longitudinal), A, represents the areas of the tissue faces, and li represents the deformed lengths measured from the central markers. Principal extensions, li, are calculated from the central marker positions that were measured from digitized images from the video recordings and are defined as the following:

\[
\frac{l_i}{l_u} = \lambda_i
\]

where li is the undeformed reference length at zero force in the ith stretching direction. The cross-sectional areas, Ai, are the deformed areas of the faces and, from incompressibility, are related to the initial areas, Ai, by the following:

\[
A_i = \frac{l_u}{l_i} A_i
\]
Cauchy stresses, $\sigma_i$, are measured forces per deformed area and, from Equation 2, are defined by the following equation:

$$\sigma_i = \frac{F_i}{A_i} = \frac{F_i}{A_{st}}\lambda_i$$

Note that for the rest of this article, stress is given in units of grams per centimeter squared to conform with present medical literature (1 g/cm$^2$=98.1 P).

Constitutive equations detail the relation between stress and strain under generalized loading conditions. Because it is well known that myocardium, as well as most soft tissue, undergoes finite strains, constitutive equations must be derived from large deformation continuum theories. Equation 4 shows the constitutive relation used for this study (details of the derivation are given in the "Appendix"):

$$\sigma_i = \mu[\lambda_i^{k_i} - \lambda_i^{k_s}]$$

where $K_s$ equals $(k_i + k_s)$ divided by 2. The parameters $\mu$ and $k_i$ estimate the characteristic material properties of the tissue, where $\mu$ is a multiplicative constant with units of stress, and $k_i$ is the dimensionless power-law constant in the $i$th stretching direction and determines the nonlinearity of the material.

Stress-extension curves were constructed from the biaxial stretching cycles and then fit to the above constitutive equation (Equation 4). Fitting was done with a modified Levenberg-Marquardt technique. Using the estimated material parameters ($\mu$ and $k_i$) and the constitutive equation, predicted stresses were calculated for exact 10%, 15%, and 20% equibiaxial extensions. An ANOVA with the Bonferroni correction was performed using the calculated (predicted) stresses as the dependent variable and percent extension, infarct or remote myocardium, and time after infarction as independent variables. Individual groups were compared using a two-tailed $t$ test. Significance was set at $P<.05$, and all data were mean±SEM except where stated. In addition, tissue anisotropy was tested by comparing the estimated material parameters with directional orientation ($k_i$ and $k_s$) as dependent variables and infarct age and myocardial location as independent variables. All statistical analyses were performed using SYSTAT (SYSTAT for WINDOWS, version 5.01, SYSTAT, Inc).

**Histology and Hydroxyproline Assay**

The histology of ovine anteroapical infarctions has been previously reported.4 For this study, infarcted tissue from three sheep harvested 6 weeks after infarction was fixed in Formalin, prepared for histology, and stained with hematoxylin and eosin.

Full-thickness samples adjacent to both the remote and infarcted samples were assayed for the amino acid hydroxyproline to determine collagen content of the healing infarct. The technique was a modification of that described by Edwards and O’Brien15 and used the standard addition method.

**Results**

Average transport time for tissue samples was 9.6±1.7 minutes; time from excision to the first equibiaxial stretching was 32.6±3.4 minutes. Samples were stretched sequentially, and the stretching protocol for each sample took approximately 30 minutes.

**Histology and Hydroxyproline Content**

Fig 3 illustrates the histology of a 6-week anteroapical ovine infarction. The predominant tissue is collagen, but isolated groups of muscle cells and vessels remain within the fibrous wall.

The effect of infarct age and aneurysm development on hydroxyproline content is seen in Fig 4. As expected, hydroxyproline content steadily increased in the apical infarct as the aneurysm developed. Hydroxyproline
content in the infarct samples significantly increased from 9.05 ± 0.15 μg/mg before infarction to 69.67 ± 7.6 μg/mg at 6 weeks after infarction, which is 7.8 times the control value. Hydroxyproline content in the basal region did not significantly deviate from control (6.9 ± 0.9 μg/mg in the control samples to 9.1 ± 1.3 μg/mg in the 6-week samples).

Sample Stiffness as a Function of Time

One experiment was dedicated to studying the effects of time on the passive stiffness of myocardium. A sample from a noninfarcted heart was mounted in the biaxial stretcher. Time from excision to the onset of stretching was 33 minutes. The sample underwent 12% equibiaxial extensions every 10 minutes for 2 hours; each stretch was preceded by 10 cycles of preconditioning. The peak stress initially was 39.77 g/cm². Two hours later the stress had increased to 49.13 g/cm², which is a 12% increase over a 2-hour span. The resulting stress-extension plots are shown in Fig 5. Despite the increase in peak stress, the curves are almost superimposable.

Thickness of the Ventricular Wall

Five within-sample measurements of each sample had mean values that ranged from 5.49 ± 0.13 mm to 14.6 ± 0.24 mm between samples and standard errors that ranged from 0.13 mm (lowest) to 0.63 mm (highest). Table 1 shows the mean ventricular wall thickness of all samples at each infarct age. There was no significant difference between the basal sections and the apical sections in control hearts. There was progressive thinning in the apical infarct as the anterioapical aneurysm developed. In 6-week animals, apical thickness decreased by 32% from control thicknesses. Remote, basal sections thickened acutely 4 hours after infarction (a significant increase of 32%) and then gradually thinned but did not return to preinfarction values.

### Table 1. Tissue Sample Thicknesses in Basal and Apical Samples at Varying Times After a Transmural Anterioroapical Infarction

<table>
<thead>
<tr>
<th>Infarct Age</th>
<th>Basal Section Thickness, mm</th>
<th>Apical Section Thickness, mm</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.38 ± 0.51</td>
<td>10.61 ± 0.53</td>
<td>NS</td>
</tr>
<tr>
<td>4 Hours</td>
<td>13.72 ± 0.47*</td>
<td>9.44 ± 0.87</td>
<td>.013</td>
</tr>
<tr>
<td>1 Week</td>
<td>12.60 ± 0.48*</td>
<td>8.15 ± 0.40†</td>
<td>.000</td>
</tr>
<tr>
<td>2 Weeks</td>
<td>12.03 ± 0.77</td>
<td>8.38 ± 0.74</td>
<td>.009</td>
</tr>
<tr>
<td>6 Weeks</td>
<td>11.90 ± 0.41*</td>
<td>7.18 ± 0.34†</td>
<td>.000</td>
</tr>
</tbody>
</table>

P values compare the basal (remote) samples with apical samples at similar infarct ages using two-tailed paired t tests; significance P < .05.

*Significant differences (P < .05) of the remote, basal samples compared with basal control samples.

†Significant differences (P < .05) of the infarcted samples compared with apical control samples.

### Tissue Stretching

Typical stress-extension curves fitted with the constitutive equation, Equation 4, are shown in Fig 6. This particular tissue sample is from a remote section of a 6-week infarcted heart. This sample is clearly anisotropic—the sample is stiffer in the longitudinal direction. Longitudinal and circumferential stress-extension data were simultaneously fit using a modified Levenberg-Marquardt technique.17 For this plot, μ = 0.488 g/cm², kₗ = 34.99, and k₅ = 29.99. Comparable fits were obtained in all stretched samples. The material parameter kₗ > k₅ gives a measure of increased stiffness in the longitudinal direction and an indication of tissue anisotropy.

Results from fitting all the stress-extension curves to the power-law constitutive equation, Equation 4, are summarized in Table 2. The material parameters, μ, kₗ, and k₅, are displayed for remote and infarcted sections for the various infarct ages. Parameters are given for 10%, 15%, and 20% equibiaxial stretches. No data are presented for 20% stretches of infarcted tissue because...
such large stretches in stiff infarcts often produced forces exceeding the range of the force transducer. 

**Stiffness as a Function of Age**

Comparisons of Cauchy stresses for each direction for remote and infarcted myocardia as a function of infarct age are shown in Table 3. For a 15% equibiaxial stretch in the remote samples, the percent change in stresses, relative to control values, is shown in Fig 7A. The data indicate that stresses increase immediately (4-hour group), peak around 1 week, and then decrease with infarct age. The circumferential direction of the remote sections becomes significantly stiffer in the 4-hour group compared with control samples. Interestingly, longitudinal stiffness of remote sections does not change significantly from control 4 hours after infarction. At 1 week, the stress levels are significantly greater than control in both stretching axes. At 2 weeks, stresses in both directions decrease from 1-week values. Finally, at 6 weeks, there is no significant difference in stresses from control (Table 3).

Percent change of stresses relative to control with 15% equibiaxial stretches in the infarcted, apical sections are shown in Fig 7B. Stresses in infarcted samples increase immediately after infarction, peak around 1 to 2 weeks after infarction, and decrease 6 weeks after infarction (Table 3). Stresses in both directions increase immediately after infarction, peak around 1 to 2 weeks after infarction, and decrease 6 weeks after infarction (Table 3). Stresses in both directions increase within 4 hours, but the increase is greatest in the longitudinal direction. Both directional stresses continue to rise, with

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**Table 2. Material Parameters for the Power-Law Constitutive Equation for 10%, 15%, and 20% Equibiaxial Stretches**

<table>
<thead>
<tr>
<th>Infarct Age</th>
<th>Stretch</th>
<th>Remote Sections</th>
<th>Infarcted Sections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>μ</td>
<td>kC</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>1.29±0.42</td>
<td>21.15±2.67</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.21±0.44</td>
<td>20.00±1.64</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.73±0.23</td>
<td>22.01±1.00</td>
</tr>
<tr>
<td>4 Hours</td>
<td>10</td>
<td>2.89±0.42</td>
<td>23.68±0.33</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>2.99±0.67</td>
<td>20.00±2.00</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>1.54±0.33</td>
<td>21.66±2.66</td>
</tr>
<tr>
<td>1 Week</td>
<td>10</td>
<td>2.96±0.43</td>
<td>24.63±2.31</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>2.40±0.18*†</td>
<td>22.64±1.45</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>2.58±0.40</td>
<td>20.05±1.80</td>
</tr>
<tr>
<td>2 Weeks</td>
<td>10</td>
<td>1.51±0.38</td>
<td>19.65±2.10</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.08±0.26†</td>
<td>22.75±2.04†</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.79±0.20</td>
<td>20.01±2.08</td>
</tr>
<tr>
<td>6 Weeks</td>
<td>10</td>
<td>1.81±0.41</td>
<td>16.43±2.15</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.17±0.31</td>
<td>18.97±1.89</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.64±0.51</td>
<td>23.88±2.40</td>
</tr>
</tbody>
</table>

Remote and infarcted region material properties, μ, kC, and kL, are presented for preinfarct, 4-hour, 1-week, 2-week, and 6-week infarct samples. Paired comparisons with the Bonferroni correction were made for 15% stretches.

*Value significantly differs from control, P<.05.
†Value significantly differs between remote and infarcted myocardium at the same age, P<.05.

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**Table 3. Cauchy Stresses (g/cm²) for Remote and Infarcted Myocardia as a Function of Infarct Age From 15% Equibiaxial Stretches**

<table>
<thead>
<tr>
<th>Infarct Age</th>
<th>Remote Myocardium</th>
<th>Infarcted Myocardium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>σC</td>
<td>σL</td>
</tr>
<tr>
<td>Control</td>
<td>19.3±7.2</td>
<td>24.3±7.6</td>
</tr>
<tr>
<td>4 Hours</td>
<td>48.5±11.5</td>
<td>31.4±10.2</td>
</tr>
<tr>
<td>1 Week</td>
<td>59.8±6.7‡</td>
<td>111.5±23.4‡</td>
</tr>
<tr>
<td>2 Weeks</td>
<td>25.3±5.3‡</td>
<td>47.4±18.7</td>
</tr>
<tr>
<td>6 Weeks</td>
<td>16.5±4.4</td>
<td>30.8±8.5</td>
</tr>
</tbody>
</table>

σC indicates circumferential stress; σL, longitudinal stress.
*P<.01 unpaired t test with Bonferroni’s correction compared with control values.
†P<.05 paired t test with Bonferroni’s correction comparing remote with infarct circumferential stresses or remote with infarct longitudinal stresses.
‡P<.05 paired t test with Bonferroni’s correction between σC and σL within the same samples.
§P=.07 paired t test with Bonferroni’s correction between σC and σL within the same samples at 4 hours.
Longitudinal stresses peaking at 1 week and circumferential stresses reaching maximum at 2 weeks. At 6 weeks, longitudinal stresses do not differ from control; however, circumferential stresses remain higher than preinfarction measurements.

Comparison of Circumferential and Longitudinal Stresses

The directional stress ratio, $\sigma_l/\sigma_c$, which is a measure of anisotropy in the stretching directions, is plotted as a function of infarct age in Fig 8. Comparison of stresses in the two stretching axes for a 15% equibiaxial stretch is shown in Fig 9. In the remote samples, anisotropy was seen 1 week after infarction, with the longitudinal stresses being greater than the circumferential stresses. In the infarcted samples, the longitudinal stresses were greater than the circumferential stresses up until 2 weeks after infarction, when the circumferential stresses became greater.

Tissue anisotropy was also tested by comparing the estimated material parameters, $k_c$ and $k_l$, with directional orientation (Table 2). Neither the basal nor apical samples behaved in an anisotropic manner before infarction. The parameter $k_l$ was not significantly greater than $k_c$ until 6 weeks after infarction. The infarcted sample became significantly anisotropic shortly after infarction. $k_c$ was significantly greater than $k_c$ at 4 hours and 1 week. $k_c$ was significantly greater than $k_c$ at 6 weeks.

Comparison of Stresses in Remote and Infarcted Tissue

The effects of anteroapical infarction on circumferential and longitudinal stresses for remote and infarcted
myocardia are shown in Fig 10. In normal (control) ovine hearts, stresses in the circumferential direction in apical samples are the same as in basal samples; however, longitudinal stresses in the apical region are approximately double basal stresses (Table 3). With aneurysm development, both directional stresses in infarcted regions progressively increase more than directional stresses in the remote regions. However, 6 weeks after infarction, longitudinal stresses in infarcted samples are similar to longitudinal stresses in noninfarcted, apical samples. In contrast, circumferential stresses in infarcted myocardium at 6 weeks remain higher than circumferential stresses in noninfarcted myocardium.

**Discussion**

This study describes the first measurements of material properties from biaxial testing of myocardium after acute infarction and presents unexpected findings: (1) the infarcted ovine myocardium undergoes an initial period of mechanical stiffening that peaks at 1 to 2 weeks and then is followed by a subsequent period in which stiffness is reversed to preinfarction levels by 6 weeks; (2) infarct stiffness does not correlate with hydroxyproline content; (3) infarctions show changes in tissue anisotropy during the healing period; and (4) the mechanical properties of noninfarcted remote myocardium also change during the remodeling period. Although the data may be specific for sheep, the findings help explain the progressive ventricular dilation and deterioration in function observed in some patients after acute myocardial infarction.

**Infarct Stiffness**

Four hours after coronary arterial ligation, the infarct is less extensible in both the circumferential and longitudinal directions. This increased stiffness may be due to myocardial edema. George18 has shown that passive stiffness is quantitatively related to the degree of myocardial edema. Interstitial edema occurs during the first few hours after infarction. Vokonas et al20 and Pirzada et al21 have reported that the initial aneurysmal bulging diminishes beginning at 1 hour and further decreases for the next 5 hours. However, in some patients with anterioapical infarctions, bulging is progressive.22,23

Previous uniaxial measurements of infarct stiffness in rabbit myocardium show a progressive increase in myocardial stiffness at 1 to 3 weeks after infarction.5,24 Our mechanical testing was performed over a much longer postinfarction period, so our initial stiffening period roughly corresponds to these studies. However, the stiffness reversal period lies beyond the duration of the studies in rabbits.

In the study by Parmley et al9 of resected ventricular aneurysms and autopsy material, they observed that mature fibrous ventricular aneurysms were seven to eight times stiffer than fibromuscular aneurysms and normal myocardium. This observation suggests that the number of viable muscle fibers in the aneurysm may affect the material properties of the aneurysm late in the healing period and afterward. Healed human infarctions vary considerably in the ratio of fibrous to muscular tissue and probably vary considerably in material properties, as the study by Parmley et al suggests. Sheep anterioapical infarctions consistently evolve into fibrous aneurysms but also contain a mixture of collagen and isolated groups of muscle strands.2,4,24 The ratio of collagen to muscle tissue probably affects both material properties and anisotropy of the infarct; however, more investigation is needed to establish this.

Previous studies indicate a direct correlation between infarct stiffness and the hydroxyproline content of the infarct. We also find a progressive increase in hydroxyproline content during the initial period after coronary arterial ligation. However, as the infarct becomes more compliant, hydroxyproline content, a reliable marker for collagen content, progressively increases. This paradoxical relation underscores the conclusion that infarct stiffness is not related to collagen content alone. Stiffness probably is also affected by collagen cross-link density25 and arrangement of collagen bundles.26-28 Caulfield and colleagues3 found that the “normal” collagen matrix was replaced by a complex “abnormal” distribution of collagen after myocardial infarction. The reduction in stiffness in late chronic infarcts has been seen in other contexts. Connelly et al8 found that “late” reperfusion, 3 hours after infarction, led to a 50% reduction in tensile strength at 3 weeks without any change in hydroxyproline content. These investigators also found a significantly reduced collagen cross-link density in a late-reperfusion group and suggested that this might also be a determinant of tissue stiffness.
Other investigators observed that ventricular stiffness decreased in growing rats (1 to 8 months), even though there was an increase in connective tissue during this period.29 Similar findings have been reported in studies of open-wound healing, where the tangent modulus (indicative of stiffness) was found to increase initially but eventually decrease within 180 days.30 They hypothesized that this extensibility was due to sliding of collagen bundles past one another.30

Anisotropic behavior of infarcted ovine myocardium reverses within the first 2 weeks. Until then, myocardium is always more stiff in the longitudinal direction than in the circumferential direction. After the second week, myocardium is stiffer in the circumferential direction. Static analysis of axisymmetrical pressure vessels indicates that the circumferential direction must withstand greater tension than the longitudinal direction. The late change in infarct anisotropy is particularly intriguing because a number of experiments suggest that the pattern of collagen deposition in the infarct depends on the pattern of stress seen in the infarct.31 If so, this collagen realignment may produce the observed anisotropic behavior.

The remote (basal) region also undergoes stiffness reversal during the healing period. There is a transient rise in stiffness in both circumferential and longitudinal directions, which peaks at 1 week and returns to control by 6 weeks. Table 1 shows that remote wall thickness transiently increases after infarction and follows the same time course as remote wall stiffness changes. The reason for the wall thickness increase at 4 hours is uncertain. Possible explanations include contracture, hypertrophy, edema, hyperemia resulting in increased residual blood volume, and an inelastic effect resulting from the altered geometry of the aneurysmal ventricle. Contracture is an unlikely cause because tissue thickness was measured approximately 15 minutes after cardiac arrest in diastole. Hypertrophy is also unlikely because of the short time interval after infarction.32-34 However, hypertrophy, if present, can explain changes in basal wall stiffness. Jalil et al35 reported that pressure-overload hypertrophy leads to an increase in fibrillar collagen and passive stiffness within 8 weeks in rats.

Edema, increased blood volume, or both can also explain increased wall thickness and stiffness at 4 hours,36 but there is no local cause for either in remote myocardium. Finally, it is possible that altered geometry of the infarcted ventricle, specifically the presence of the thinned infarction, changes loading conditions (stresses) in the basal region. Conceivably, these changes may allow inelastic shortening of the wall, sometimes referred to as “reverse creep.” This shortening would increase wall thickening.

Implications of Infarct Stiffness

Based on previous uniaxial measurements of infarct compliance, stiffness was believed to progressively increase throughout the healing process. Using data from these uniaxial mechanical tests, Bogen et al37 previously simulated the effect of the developing aneurysm on the left ventricle in an isotropic, initially spherical membrane model of the infarcted ventricle. Computations based on finite element solutions of the membrane model yielded diastolic and systolic pressure-volume relations. The computer model predicted that the acutely infarcted left ventricle would have the highest borderzone stress and extension, the most compliant ventricle, and the lowest end-systolic pressure-volume relation. The chronic (inextensible) aneurysms were characterized by lower borderzone stress and extension, the least compliant ventricle, and an end-systolic pressure-volume relation that was near normal. Overall, left ventricular performance was predicted to improve with time. However, with these new biaxial stress-extension data, the model of Bogen et al37 would predict increasing borderzone stress and extension and worsening ventricular performance as compliance of the infarct begins to increase approximately 2 weeks after coronary arterial occlusion.

The changes in compliance and anisotropic behavior of infarcted myocardium during the healing period indicate a dynamic role of the infarct in the remodeling process and in ventricular performance. Acute infarction subjects the heart to an immediate increase in mechanical load that is partially compensated by the Frank-Starling mechanism in nonischemic myocytes.2,38 Diastolic wall stresses increase severalfold39,40 and cause cavitary dilation and eccentric hypertrophy.41 There is slippage between nonischemic myocytes,39 and release of circulatory and neuroendocrine transmitters stimulates hypertrophy of viable cells.41 Increased infarct stiffness during remodeling most likely attenuates regional and global ventricular dilation and, therefore, wall stresses. Differences in direction of infarct stiffness (ie, longitudinal versus circumferential) may affect infarct deformation and wall stress and, consequently, ventricular geometry and performance. Our observation that infarct stiffness decreases after the initial rise means that infarct deformation and wall stress will increase as the infarct becomes more compliant, and performance will decrease.

The changes in material properties after acute myocardial infarction may vary between species and likely vary between patients for a variety of reasons. The sparse data available suggest that myocyte survival within the infarct and the pattern and type of collagen synthesis may be influenced by the severity of local ischemia, local stresses, local hormones and autacoids, and other factors. The resulting compliance and anisotropy of the infarct represent the sum of the biochemistry and histology of the infarct and the mechanical forces acting on the infarct and ventricle. The data provided by these studies clearly indicate that the relation between regional stresses and mechanical properties is dynamic during the healing period. More data describing the material characteristics of both infarct and nonischemic tissue at various intervals after acute myocardial infarction are needed from animal as well as human samples. Furthermore, these data require correlation with in vivo measurements of ventricular strain, histology, clinical outcome, and the morphology and biochemistry of the collagen network. Because so little is known of the relation between the biochemistry and histology of the healing infarct and the mechanical properties of the infarct at various stages during the healing process, predictions of ultimate ventricular geometry and performance for individual patients remain speculative.
Study Limitations

There are several unaddressed considerations that remain before the role of the ventricular material properties in the remodeling process after acute myocardial infarction can be understood. This study lacks in vivo data to follow changes in reference lengths, $l_{ref}$, which indicate inelastic (ie, plastic or creep) deformation during remodeling. In an earlier study, thinning and stretching of borderzone myocardium were observed after surgical plication of ovine anterioapical aneurysms. Thickening of remote myocardium 4 hours after infarction in this study raises the possibility of reverse creep. We have no data regarding changes in viscoelastic properties of borderzone and remote myocardia and no data regarding material properties of myocardium during systole. Although changes in regional ventricular wall stresses are widely thought to drive the remodeling process, we did not estimate regional stresses and therefore cannot comment on the relation between regional stress distribution and the observed changes in material properties. Last, ovine infarcts must be more systematically defined histologically. The ovine infarct, like the human infarct, is not homogeneous, and the mix of collagen, muscle, and supporting tissues probably varies between individual animals and patients. Changes in the architecture of the collagen network in surviving myocardium in surviving myocardium remain unknown.

Nevertheless, these studies underscore that the material properties of not only the infarct but also surviving myocardium change after coronary arterial occlusion and that these changes probably affect the remodeling process and ventricular performance. The discovery of increasing compliance in infarcted myocardium after initial stiffening implies that in some patients, increasing ventricular wall stresses produced by remodeling are not relieved by transitory infarct stiffening. Thus, this study suggests that infarct healing may not sufficiently counteract the forces producing ventricular dilatation.

Inferences

Recognition of the dynamic changes in the mechanical properties of the healing infarct introduces the possibility of interventions. Pharmacological or surgical methods (eg, cardiomyoplasty) to increase infarct stiffness may favorably alter the remodeling process and better preserve ventricular function. For example, angiotensin-converting enzyme inhibitors improve survival after myocardial infarction, but the mechanism probably is more complex than simple reduction of afterload. It is possible the drug alters the mechanical properties of the healing infarct as well. Reperfusion may reduce the size of the infarct even if too late to rescue myocytes, and this may be due to altered mechanical properties of the necrotic myocardium. These and other possible interventions directed toward stiffening the infarct during the healing process may eventually prevent the late ventricular dilatation and functional deterioration that occur in some patients after acute myocardial infarction.

Appendix

Constitutive equations describe the relation between stress and strain under generalized loading conditions. Because it is well known that myocardium, as well as most biological soft tissues, undergoes finite strains, strain energy constitutive equations must be derived from large deformation theories. The fundamentals of large deformation can be found in many monographs. Typically, soft tissue has been considered a hyperelastic, incompressible material. A hyperelastic material has properties that relate a strain energy function to work done by imposed stresses. Presented below is a brief description of the constitutive equation and a form of the strain energy function derived from a phenomenological approach. Gupta provides a more in-depth discussion of the derivation of the constitutive relation used in this study.

For a hyperelastic material, or Green elastic solid, it is assumed that there exists a strain energy function per unit volume, $W$, that is solely dependent on $F$, where $F$ is the deformation gradient tensor. It is further postulated that the rate of change of the strain energy is totally accounted for by the stress power. Thus, the constitutive equation for an anisotropic, incompressible, hyperelastic material takes the form

$$
\sigma = -p\delta + f(F)
$$

where $\sigma$ is the Cauchy stress tensor, $p$ is an arbitrary uniform hydrostatic pressure determined by boundary conditions, $\delta$ is the Kronecker delta, and $f$ is a single-valued function of $F$. Because our biaxial mechanical tests involve homogeneous deformations in which the principal directions are aligned with the circumferential and longitudinal material directions, this constitutive equation can further be reduced to a more convenient form. Within the framework of the plane stress assumption (if the sample is stretched in the plane perpendicular to axis 3, then $\sigma_3=0$), the hydrostatic pressure can be solved for, and the remaining principal stresses, $\sigma_1$ and $\sigma_2$, can be written as the following:

$$
\sigma_1 = \frac{\partial W}{\partial \lambda_1} = \lambda_1 \sigma_1, \quad \sigma_2 = \frac{\partial W}{\partial \lambda_2} = \lambda_2 \sigma_2
$$

Once the form of the strain energy function is specified, stresses can easily be calculated from Equation 6. It is well known that passive myocardium, as well as most soft tissue, is highly nonlinear. Typically, nonlinear strain energy functions are given in terms of functions of the invariants of some deformation tensor. It may be more convenient, however, to write the strain energy function in terms of the principal extensions, $W=W(\lambda_1, \lambda_2, \lambda_3)$. A generalized form is the following:

$$
W = \sum_{\eta} \mu_\eta \left[ \lambda_1^{\eta_1} \lambda_2^{\eta_2} \lambda_3^{\eta_3} - 3 \right]
$$

where $\mu_\eta$ and $\lambda_\eta$ are constants. For an incompressible material, $\lambda_1\lambda_2\lambda_3=1$ and therefore $\lambda_3=1/\lambda_1\lambda_2$. Variations of this form have been developed by Blatz et al while working on biological material and Ogden while working with rubber. However, a single term from the series in Equation 7 provides an adequate description of normal and infarcted myocardium.

The above form of the strain energy function suggests another strain energy function for anisotropic materials:

$$
W = \mu \left[ \lambda_1^{k_1} \lambda_2^{k_2} \lambda_3^{k_3} - 3 \right]
$$

where $k_\eta$ is an independent power term, $k_\eta$ for each stretching axis. Here it is assumed that the material is orthotropic and that the material axes and stretching axes are
aligned. In this case, the material extensions and principal extensions are identical. Again, if a rectangular tissue sample undergoes biaxial testing and we invoke plane stress assumption, the principal stresses become the following:

\[ \sigma = \mu(\lambda_k^4 - \lambda_3) \]

where \( \lambda_k \) equals \( (\lambda_1 + \lambda_3) \) divided by 2. This is the form of the constitutive relation used in this study.

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