Relationship between passive tissue strain and collagen uncoiling during healing of infarcted myocardium

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Abstract

Objective: The structure of the collagen scar during healing of a myocardial infarction is a determinant of the function of the remodeled tissue. We hypothesize that the passive deformations of both scar and normal tissue are related to the underlying collagen uncoiling as the tissue stretches, and that the unloaded tortuosity of the collagen may be a determinant of tissue stiffness at low ventricular pressure. Hence collagen uncoiling and tissue strain were measured during passive loading in normal tissue, and in healing infarct tissue.

Methods: Left ventricles of rats were infarcted by ligation of the left anterior descending artery for 2 weeks. Surface strains were measured during passive inflation in the scar region in one set of excised hearts, and other arrested hearts were fixed at different ventricular pressures, after which collagen tortuosity was measured in the infarcted and normal tissue.

Results: Passive loading strains were smaller in the scar in both the fiber and cross-fiber directions. Tortuosity decreased with load in normal and infarcted tissue, with fibrils tending to straighten more in the scar tissue at higher pressures (1.056 ± 0.009 vs. 1.024 ± 0.009 at Ps = 20 mmHg) with similar tortuosities at zero pressure (1.110 ± 0.012 vs. 1.098 ± 0.019). The decrease in tortuosity with strain was greater for the infarcted tissue.

Conclusions: The greater stiffness of infarcted tissue at low pressure is not due to ‘straightened’ collagen fibers, and there may be a different three-dimensional structure of infarct vs. normal coiled collagen fibers which can affect the material properties of these tissues.

Keywords: Extracellular matrix; Rat, heart; Scar tissue; Tortuosity; Mechanics; Collagen

1. Introduction

During healing of a myocardial infarction, cardiac function is determined by the mechanics of both the scar region and the adjacent contracting myocardium. The composition of infarcted myocardium during post-infarction remodeling is very different from that of normal heart tissue. In normal myocardium, collagen comprises only 2–5% of the tissue [1], but during scar formation in the infarcted region, collagen content continually increases for several weeks or months, reaching a plateau with dramatic increases in collagen content [2,3]. In a study of rat myocardium following 13 weeks of infarct, the infarcted region of the left ventricular (LV) free wall was found to contain up to 6 times more collagen than in control rats [4]. Other studies have indicated a high area fraction of collagen in the post-infarction scar tissue, which may be 70% or higher [5]. The largest changes in ventricular collagen structure usually occur during the first few weeks following infarction in the rat. Fishbein et al. [2] found that scar formation was ordinarily complete in the rat 21 days after coronary occlusion, and indices of the evolution of the infarct (infarct thickness, volume of the infarct, surface area of infarct) were close to their final, steady-state values by 13–15 days.

Although collagen content increases throughout the healing process, infarcted tissue stiffness peaks between 1 and 2 weeks post-infarction [6], and chamber stiffness has been shown to decrease with time after infarction in the rat [7]. Peak rupture time can vary from 1 week [8] to 4 weeks [9] post-infarction. Collagen cross-linking also increases in the scar during remodeling, which can alter the mechanical properties of the remodeling tissue [4,10]. The arrangement...
of the collagen fibers in the scar will also be a determinant of passive stiffness since the scar is anisotropic [11]. Although the diastolic pressure–volume relationship of the ventricle is known to change in the infarcted heart [12], the local regional mechanics are more directly related to the local material properties. Both normal and infarcted myocardium exhibit non-linear stress-strain behavior during passive inflation, but local deformations will be different in the infarct and non-ischemic regions. Although the material properties of the normal tissue may be attributed to both collagen and myocytes, in the scar region constitutive properties must be a consequence of the extracellular matrix structure since the material in this region is composed primarily of collagen.

It has been shown previously in the normal rat heart that as the ventricle distends during passive inflation, the perimysial collagen fibers become less coiled, and the straightening tends to reach a limit in parallel with lengthening of the sarcomeres [13]. Since scar tissue resulting from infarction also contains coiled collagen fibers, it is possible that the uncoiling in the infarct is different from that in the normal tissue. These differences may be directly related to the material properties and regional deformations of these dissimilar tissues. Our hypothesis was that the increase in passive stiffness in infarct tissue was due not only to the obvious increase in collagen content, but also to ‘straightened’ collagen fibers which should be much stiffer than coiled perimysial fibers in normal tissue. Thus the objective of this work was to relate the uncoiling of collagen fibers in both normal and infarcted regions of myocardium to the passive strains in these tissues. The 2-week post-infarct time point was chosen since tissue stiffness peaks around this time [5,6], and the healing process in the rat is reaching its plateau [2]. We found that uncoiling of the larger tortuous fibers during loading occurred at a higher rate in the healing scar tissue, although the tissue strain was markedly decreased at the same time. These results indicate that the high stiffness of this scar tissue at low pressures is not due to excessive straightening of collagen fibrils as originally suspected, and imply that the three-dimensional coiling pattern of collagen in the scar tissue may be different from that in normal tissue.

2. Methods

All acute and chronic animal studies were performed according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985), and protocols were approved by the UCSD animal subjects committee. A total of 48 Male Sprague-Dawley rats were used in this study, and 34 of these were used for analysis: 12 for passive epicardial strains (control and ischemia groups) and 22 for tortuosity (4 different fixation pressure groups, \( n = 5 \) or 6 in each group). Each animal was anesthetized with ketamine 100 mg/kg, morphine 2.5 mg/kg, and xylazine 5 mg/kg. (i.p.). Under positive-pressure ventilation with room air, a left thoracotomy was used to expose the LV epicardial surface. A 5-0 suture with TF-4 needle was used to completely ligate the left anterior descending coronary artery approximately 1–2 mm from its origin. The chest was closed and the animal allowed to recover. Two weeks after the initial surgery, the rats were anesthetized with sodium pentobarbital (100 mg/kg) and ventilated with air. The heart was exposed with a median sternotomy, the ascending aorta clamped, and the heart arrested with 3 ml of a hypothermic, hyperkalemic modified Krebs-Henseleit solution via direct injection through the apex into the LV. The arresting solution contained (in g/l): NaCl 4.0, KCl 4.48, NaHCO3 1.0, glucose 2.0, 2,3-butanedione monoxime (BDM) 3.0, and heparin 10 000 U/l. The heart was excised, weighed and suspended by a cannula placed in the aorta. The isolated heart setup described previously was used to obtain passive pressure–volume data [14]. Briefly, the LV was vented with a small catheter through the apex, and the right ventricle vented with an incision through the right ventricular free wall. A fluid-filled balloon attached to a second cannula was placed in the LV and secured with a purse-string suture in the mitral annulus. The balloon was connected to a Statham P23ID pressure transducer and volume infusion pump.

Pressure–volume data were acquired by inflating the balloon at a rate of 1 ml/min to a peak pressure of 25–30 mmHg, followed by a deflation to the original volume. After 3 preconditioning cycles, pressure and volume were sampled at 25 Hz during the loading portion of a cycle using an analog-to-digital converter, 486 computer and data acquisition software. In two groups of hearts (normal and infarct), two-dimensional strains were measured on the left ventricular epicardial surface. A triangle of white dots was created at the mid-ventricle with titanium oxide powder mixed with water applied to the epicardium. The locations of the dots were then recorded with a video camera and macro lens during the same volume-infusion cycle used for pressure–volume data. Following the mechanical data acquisition, the coronary circulation was perfused with India ink via retrograde perfusion through the aortic cannula to verify that the surface markers were completely within the infarct (non-stained) area in the infarct group of hearts.

Pressure–volume curves were fitted by least squares to third-order polynomials, and volumes found at equal pressure increments from 0 to 25 mmHg. Strains were referred to a local cardiac coordinate system defined at the centroid of the 3 markers. Two-dimensional finite Lagrangian strains referred to the zero-pressure state were computed during the loading portion of the cycle [14]. The two-dimensional strain tensor was calculated for each individual heart. Each strain component was fitted with a third-order polynomial so that strains could be found at matching pressure increments. The 3 strain components in cardiac coordinates...
were averaged at 5 ventricular pressures. In each individual heart, strains were also computed in a fiber coordinate system. Thus the strains were rotated through the measured epicardial collagen fiber angle. For the infarct hearts, the most epicardial collagen angle was within 10% of the epicardial surface.

Histologic examination of the tissue included quantification of collagen tortuosity in normal and infarcted left ventricular myocardium from the same animal, collagen fiber angle in the scar, and scar size in terms of a scar/normal tissue area fraction from a cross-sectional ring near the center of the infarct. Following the pressure–volume data acquisition, the hearts to be used for tortuosity measures were fixed at one of 4 different prescribed pressures (0, 5, 10, 20 mmHg) by retrograde perfusion of 10% buffered formalin phosphate through the aortic cannula. Hearts used for strain analysis were all fixed at zero transmural pressure. After adequate fixation, several cross-sectional rings were taken from each of the infarcted hearts near the center of the infarct. Each edge of the rings was cut parallel to the local circumferential axis and used as a reference edge for collagen fiber angle measures. One of these rings was embedded in paraffin so that sections were cut parallel to the epicardium through the infarct zone. 10-μm-thick sections were cut at 100-μm intervals through the wall thickness. The infarcted zone had variable wall thickness, typically yielding 5–8 transmural sections in hearts fixed at low pressure, while in the hearts fixed at high pressures, there were 4–6 sections. The sections were cleared of paraffin, brought to water through a series of graded ethanols, and stained for 90 min in picrosirius red (0.1% sirius red F3BA in saturated picric acid) to enhance the birefringence of the fibrillar collagen.

The tortuosity or ‘waviness’ of the collagen fibers was quantified using a technique previously developed in our laboratory [13]. Briefly, the picrosirius-stained sections were rotated on the circular stage of a microscope (40× objective) under polarized light until a particular collagen fiber was best illuminated. The color image was captured onto a Macintosh computer, and using image processing software (NIH Image, version 1.49), the path of the collagen fiber and its midline period length were traced with the mouse using line segments sufficiently short that the fiber appeared smooth. The tortuosity of a fiber was defined as the ratio of total fiber length to midline length. Sections were analyzed from both normal and infarcted tissue in each heart. Tortuosity in at least 15 random fibers was measured near the center of the tissue in each section to obtain an average tortuosity at each depth.

To quantify the average orientation of the collagen fibers, the tissue was viewed under low-power magnification with polarized light through a color video system. In normal tissue, individual collagen fibers can be viewed and their orientations determined by averaging at least 5 angles in the epicardial section. In the infarct hearts used for the strain analysis, sections taken parallel to the epicardial tangent plane at 4–6 depths were examined with an optical averaging scheme. At a location near the central portion of the scar, the section was placed on a rotating stage, and the average extinction angle was defined as the angle of minimum light transmission. The total light transmission was measured as the RMS voltage of the composite video signal. The amount of light transmission was validated in several sections by computing the average brightness level with digital image analysis. The fiber angle with respect to the circumferential axis was then computed for each field based on the extinction angle and the reference edge of the section. At least 5 angle measures were taken and averaged for each transmural location.

A cross-sectional ring of tissue from the center of the infarct (at the latitude with the greatest scar width) was used to measure the relative size of the scar in each heart. Slices were embedded in paraffin and stained as described above. Low-power microscopy was used such that the entire left ventricular outline was visible in one field. The image was acquired to the computer, and the left ventricular epicardial and endocardial contours, as well as the scar borders, were digitized. Thus we were able to compute the scar/normal tissue area fraction from the cross-sectional ring near the center of the scar region.

To evaluate the effect of infarction on the tortuosity–pressure, strain–pressure and pressure–volume relationships, a two-way analysis of variance and post-hoc Fisher’s Protected Least Square Differences comparisons were performed with type of tissue (infarct/normal) and either tortuosity, strain or pressure as the independent factors.

3. Results

Of the 48 animals used, 10 of the infarcted animals died before the allotted 2-week period, and 4 of the animals were excluded because the epicardial infarct area was less than 10% of the left ventricular surface. Data were analyzed in the 27 remaining infarct animals, and 7 additional animals with matched body weights were used to obtain control strain measures.

Body weights were not different for the animals in the different groups (351 ± 29 g for all animals at the time of the study). Average heart weight was 1.57 ± 0.29 g for all animals used (infarct and normal), and again not statistically different between the groups. The shape of the non-linear pressure–volume curves was similar to those observed previously [14,15], although the relationship for the infarcted hearts was shifted to the right as expected (Fig. 1). The unloaded volume of the hearts increased from 0.045 ± 0.012 ml in the normals to 0.121 ± 0.019 ml in the infarcted hearts. A two-way ANOVA showed a statistical effect of tissue treatment (normal/infarct) on the volume (P < 0.001) and also a statistical effect of the treatment on the change in volume with pressure (P = 0.036).

Surface strains were found in the infarcted region dur-
Fig. 1. Mean pressure–volume curves (± s.d.) for both infarct (n = 27) and control (n = 7) rats during the infusion portion of the loading cycle. Volumes include the volume of the balloon material and cannula inside the left ventricle. As expected, the pressure–volume curves of the infarcted hearts are shifted to the right, and were statistically different from the controls. Thus the thinning of the infarcted portion of the wall leads to increases in the unloaded ventricular volume, and overall decreases in ventricular compliance.

Fig. 2. Mean in-plane epicardial strains during passive inflation of the left ventricle. Strains were referred to the cardiac coordinate system determined in the fixed tissue. In the normal tissue, the two normal strains ($E_{11}$, circumferential; $E_{22}$, longitudinal) are positive corresponding to stretching, and the shear strain ($E_{12}$) is negative due to the left-handed twist. In the infarct area, statistical differences were found in $E_{11}$ and $E_{22}$ compared to control regions, both decreasing after infarct for the same pressure load, indicating a much less compliant tissue.

ing passive inflation and compared to surface strains from normal control hearts. The magnitude of each strain component in cardiac coordinates decreased in the scar region (Fig. 2). Circumferential strain ($E_{11}$) remained positive with a smaller magnitude after infarct, while the longitudinal strain ($E_{22}$) was very close to zero in the infarcted region. Shear ($E_{12}$) remained negative, indicating that the scar region twists in the same left-handed fashion as the normal left ventricular myocardium during diastolic inflation. ANOVA showed a statistical effect of tissue type (infarct/control) on the circumferential ($P = 0.035$) and longitudinal ($P = 0.002$) components, but not on the in-plane shear component.

In order to examine the local deformations with respect to the underlying tissue structure, the passive strains were transformed into a local fiber coordinate system based on the epicardial collagen fiber direction at the location of the local strain measurement. Fig. 3 shows these fiber strains for both the infarct and normal hearts. There was a statistical effect of tissue type (normal/infarct) on each of the 3 strain components in this coordinate system ($P = 0.006$, $P = 0.034$, $P = 0.003$ for ff, cc and fc, respectively), as well as a significant effect of tissue type on the change in strain with pressure (interaction, $P < 0.02$ in all cases). The fiber and cross-fiber strains were closer to zero after infarction, indicating very small deformation along these axes on the epicardium. The fiber/cross-fiber shear was negative in the scar tissue, indicating that shearing between collagen fibers in the scar is different from fiber shear in normal tissue.

The volumes of the infarcted ventricular cavities, and thus the ventricular radii, were substantially increased compared to control hearts. The average infarct area in all of the infarcted hearts was $32 \pm 14\%$ of the left ventricular cross-sectional area near the center of the infarct. There was no statistical effect of pressure group on this scar/normal tissue area fraction ($P = 0.245$); the mean values were $34 \pm 15\%$, $30 \pm 12\%$, $28 \pm 7\%$, $26 \pm 13\%$ and $44 \pm 17\%$ for the tortuosity ($P = 0$, 5, 10 and 20 mmHg, respectively) and strain groups. The orientation of the collagen fibers across the wall (Fig. 4) changed substantially from a normal distribution. In normal tissue, the perimysial collagen fibers tend to align with the myocytes, thus showing a variation of roughly $100^\circ$ from epicardium to endocardium [16]. After 2 weeks of ischemia and remodeling, the transmural gradient of collagen orientation tended to disappear. The direction in the epicardial-tangent plane varied from $-14^\circ$ at $21\%$ of the distance from epicardium to endocardium, to $-28^\circ$ at $66\%$ of the wall thickness. Thus the greatest remodeling in this angle was seen in the inner portion of the wall, where normal collagen angles are positive.
Fig. 3. Mean in-plane fiber strains, found by rotating the strains of Fig. 2 through the local collagen fiber angle. There was a significant effect of type of tissue (infarct/normal) on each of the 3 strain components. In this local coordinate system, strain transverse to the long axis of the collagen fibers was very close to zero in the infarcted tissue, and strain along the fibers decreased substantially also. $E_{fs} =$ fiber strain; $E_{cc} =$ cross fiber shear.

Fig. 4. Orientation of collagen fibers across the ventricular wall within the scar. Measurements were grouped into 3 transmural locations, and each individual measurement represents the average angle in that section found using video averaging techniques. Previous studies have shown that the collagen angle in normal tissue closely follows the local muscle fiber angle. The collagen angle in the infarct showed a much lower transmural variation compared to normal tissue, with the greatest difference occurring near the endocardium.

In both normal and infarcted tissue, there was a significant effect of pressure on the tortuosity as expected, with the fibers becoming straighter at higher pressures (Fig. 5). The tortuosities were averaged for all transmural regions in each heart (Fig. 6). There were no transmural variations in tortuosity in the scar, and in the control sections, tortuosity tended to be smaller towards the epicardium as previously reported [13]. The change in tortuosity of the collagen

Fig. 5. Sample micrographs of picrosirius-stained tissue viewed with brightfield microscopy ($400 \times$). (A) Normal tissue fixed at 0 mmHg; (B) normal tissue fixed at 20 mmHg; (C) infarcted tissue fixed at 0 mmHg; (D) infarcted tissue fixed at 20 mmHg. Note the decrease in tortuosity of the dark-stained collagen fibers in the high pressure state, and the increased collagen density in the infarcted tissue.

Fig. 6. Collagen fiber tortuosity as a function of pressure in normal and infarcted tissue. Each point represents measurements from a group of hearts fixed at the indicated loading pressure. In both types of tissue, tortuosity decreased as the pressure increased, indicating a straightening of the fibers with load. The change in tortuosity was similar at the low pressures, but above 10 mmHg the collagen fibers in the infarcted tissue uncoiled more as shown by the smaller tortuosity in these hearts.
fibers with pressure was significantly affected by the type of tissue (infarct/normal), $P = 0.0265$. Individual comparisons showed that the tortuosities were significantly different only at the higher pressures ($P = 10$ and $P = 20$). Thus at higher passive inflation pressures, the collagen fibers tended to straighten more in the infarcted tissue (towards a tortuosity of 1—i.e., a straight line). Although tissue remodeling may be a function of infarct size [17], there was no significant correlation between tortuosity and scar/normal area fraction in any of the 4 pressure groups (range of $r$ values: 0.24–0.43; $P > 0.4$).

When tortuosity is plotted versus strain (Fig. 7), differences in the tissue mechanics independent of load become apparent. Again both sets of coiled collagen fibers start at nearly the same tortuosity at zero strain. In the infarcted tissue, the relative change in strain is roughly the same as the change in tortuosity. The change in strain for a two-dimensional sinusoidal ‘coil’ and a three-dimensional helix were found using kinematic relationships for these two structures [18], and are also shown in Fig. 7 as the theoretical lines. The two-dimensional theoretical line is the change in tortuosity of a sine wave with an initial tortuosity of 1.106, which becomes a straight line (tortuosity = 1) at a Lagrangian strain of 0.112. The three-dimensional theoretical line shows the sinusoidal two-dimensional projection of a helix [19,20] at zero strain (tortuosity = 1.113), and becomes completely straight at a strain of 0.244. The differences in the theoretical predictions correspond to those seen in the heart: in the normal tissue tortuosity decreases less for a given strain increment (three-dimensional prediction), while the infarcted tissue data lie closer to the two-dimensional prediction.

4. Discussion

The objective of this study was to quantify the uncoiling of collagen fibers within a healing scar during passive filling of the ventricle in order to examine the role of collagen tortuosity during the healing process after infarction. In normal tissue, coiled collagen fibers uncoil during passive loading. Compared to this normal uncoiling, the collagen fibers uncoiled more in an infarct above 10 mmHg. This corresponded to very small in-plane stretching transverse to the fibers, as well as substantially smaller collagen fiber strain. These results suggest that ‘straightened’ collagen fibers do not play a substantial role in the passive mechanics of the scar at low pressures. At higher pressures the collagen is straightener in scar tissue, consistent with a more two-dimensional configuration, which implies increased stiffness in the scar due to the straightened fibers at these pressures. Previous investigators have shown changes in the coiling of collagen fibers with load, and made implications for myocardial mechanics based on these changes [19,21]. In a recent study in normal myocardium, MacKenna et al. [13] showed that perimysial collagen fibers uncoiled and did not stretch during passive loading in normal tissue. The changes in tortuosity were similar to those found in this study in the normal tissue. Their results indicated a tight coupling between myocytes and collagen. In the present study, the myocytes are replaced by extracellular matrix after infarction, hence coupling between collagen fibers must play a major role in the mechanical properties of scar tissue. The results of the current study show that the larger coiled collagen fibers uncoil in both normal and infarcted tissue during passive loading, although the rate of uncoiling is greater in scar tissue. Since the compliance of the scar is close to zero in the direction transverse to the long axis of collagen fibers, other parts of the matrix besides the larger coiled collagen fibers probably play an important role in determining the overall material properties of the scar tissue.

Other investigators have shown altered orientation of collagen in scar tissue following an infarct. Whittaker et al. [22] showed a variation in collagen angle ($-14^\circ$ to $13^\circ$) from epicardium to endocardium in dogs after 6 weeks of coronary occlusion, and this variation is much less than the normal muscle and collagen fiber angles in the dog heart, which can vary over 100$^\circ$ from epicardium to endocardium [23]. In the present study, the change in collagen angle across the wall was also decreased from the normal collagen angle which parallels muscle fiber angle. It has been proposed that stress in the tissue may be a determinant of orientation of newly formed collagen fibers. If this is the case, the present results would indicate that stress in the scar during remodeling is fairly uniform across the wall, such that the orientation of collagen lay-down is also uniform compared to the original fiber and stress distributions. Another possibility for a transmural infarct is that the thinner wall in the scar is remaining tissue from the
epicardial portion of the wall at that location, and that the endocardial portion of the wall was lost during the remodeling process, thus eliminating most of the fibers with positive angles. The actual mechanism by which the transmural fiber angle becomes uniform remains to be discovered.

The coiled perimysial collagen fibers that run parallel to the muscle fibers appear wavy in normal tissue. Previous investigations have shown these coiled fibers resemble a three-dimensional helix [19]. The convolution index of a helix is defined as the ratio of the fiber length to the diameter of the coil. A similar relationship exists for the tortuosity of a two-dimensional wavy fiber, which is the ratio of fiber length to wavelength of the coil. The convolution index of a helix is the three-dimensional analog to tortuosity of a sine wave in two dimensions. Since the two-dimensional projection of a helix is a sine wave, the change in tortuosity, as measured in two dimensions, is less for a helix than for a two-dimensional sine wave, for a given change in mid-line length or fiber strain [18]. Thus, a three-dimensional coil will have a lower decrease in tortuosity than a two-dimensional coil given the same overall lengthening. Our results for tortuosity versus strain suggest that the perimysial collagen structure in normal tissue may be a more three-dimensional structure (e.g., a coiled helix), while the structure of similar-sized collagen fibers in infarcted scar tissue may be more two-dimensional. A planar-type fiber has been described in other tissues which contain more collagen (e.g., skin and tendon) [24], in which the crimp pattern of the individual collagen fibers has been described as two-dimensional.

The stiffness of scar tissue is much greater than normal myocardium presumably due to the substantial increase in the volume fraction of collagen. The change in stiffness could also be related to the amount of uncoiling of the collagen fibers: i.e., a straight collagen fiber is much stiffer than a coiled or wavy strand due to the bending versus tensile rigidity. Our results indicate that the collagen tortuosity in scar tissue is not different from that of normal tissue in the unloaded state, implying that the increased stiffness of the scar at low pressures is not due to ‘straightened’ collagen fibers. This is not true at higher pressures, where the increases in stiffness of infarcted tissue can be attributed not only to the higher collagen density, but also to the increased straightening of the fibers, hence increased stiffness of the individual fibers. Another factor that can affect collagen fiber stiffness is the collagen type, which has been shown to change with time in the scar [25]. Since type III collagen is not as stiff as type I, and type III content is likely to be elevated at 2 weeks, the stiffness of the scar collagen fibers may be different from that in control. This factor could affect the three-dimensional configuration of the fibers as well as the changes in tortuosity as functions of load.

Several limitations of the present study should be considered. The measures of collagen orientation in scar are not as reliable as those in normal tissue. In normal tissue individual fibers are observed and their directions measured, while in scar tissue the measurement represents an average of all tissue which exhibits light extinction at a certain angle (i.e., the overall collagen fiber angle). It is also possible to measure individual fiber angles in scar tissue in order to obtain the mean collagen angle [26]. We employed both methods in several sections and found the results to be comparable, with small exceptions near the endocardium and epicardium where variability within each field was highest. The optical averaging method was much faster; however, the individual fiber measurements provide additional information on fiber angle distribution and variation about the mean. Although the final results show a fairly consistent gradient of angle across the wall in scar, the angles are variable even in one section of tissue, which might imply more isotropic material parameters on a global tissue scale, and this variability is not quantified with the optical averaging technique.

There were other limitations of the study worth noting: components of the extracellular matrix besides the large coiled collagen fibers [27] were not examined in this study, and the remodeling of these smaller structures during scar formation has not been well characterized. Material properties transverse to the large collagen fibers will be related to the other structural components oriented in these directions which were not looked at in the present study. Although the results of this study suggest certain three-dimensional characteristics of the collagen fibers in normal and infarct tissue, we do not have direct evidence for the appearance of these structures. Further microscopic studies must be performed to validate these predictions. More thorough examination (e.g., at multiple times during the remodeling) could indicate whether the original collagen fibers were still present after 2 weeks, or if the original fibers had all been replaced by ‘new’ fibers. If many of the ‘old’ fibers were still present, they could still have the three-dimensional helical coiling pattern of normal fibers rather than the planar pattern predicted for all of the collagen in the scar.

Passive strains were only measured on the epicardial surface in these hearts, so the relationships between mechanical deformation and the changes in collagen coiling are only valid at the epicardium. Since stress, strain, muscle and collagen angles all change as a function of transmural location, more comprehensive studies are needed to look at these relationships at other locations in the wall. Also, the control data for the strain analysis were obtained from healthy animals. There may be effects of the chronic surgery (e.g., the heart adhering to the surrounding tissue) that are seen only in the infarcted hearts and not in the control strain group. These studies were performed relatively early in the infarct remodeling process. Since the scar properties are known to change with time, and collagen formation and cross-linking are not complete at 2 weeks, further studies at a later time point are needed to
describe these same relationships in fully mature scar tissue. It is also possible that edema could play a role in the increased scar stiffness, although edema usually peaks earlier than 2 weeks, and in any case should not affect the kinematic relationship between tissue strain and collagen uncoiling.

In summary, changes in tortuosity in normal and healing infarcted tissue indicate that collagen fibers are not straight in scar tissue at low pressures, hence this is not a mechanism of the increased scar stiffness near zero pressure. Since the fibers do become straight at higher pressures, even more so than in normal tissue at the same stretch, this may be an important factor during systolic contraction when ventricular pressure is high. The strain–tortuosity relationships imply a different three-dimensional configuration for coiled collagen fibers in normal versus infarct tissue, but direct morphologic examination is necessary in order to accurately describe these structural differences.

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References