Crack Propagation Versus Fiber Alignment in Collagen Gels: Experiments and Multiscale Simulation

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It is well known that the organization of the fibers constituting a collagenous tissue can affect its failure behavior. Less clear is how that effect can be described computationally so as to predict the failure of a native or engineered tissue under the complex loading conditions that can occur in vivo. Toward the goal of a general predictive strategy, we applied our multiscale model of collagen gel mechanics to the failure of a double-notched gel under tension, comparing the results for aligned and isotropic samples. In both computational and laboratory experiments, we found that the aligned gels were more likely to fail by connecting the two notches than the isotropic gels. For example, when the initial notches were 30% of the sample width (normalized tip-to-edge distance = 0.7), the normalized tip-to-tip distance at which the transition occurred from between-notch failure to across-sample failure shifted from 0.6 to 1.0. When the model predictions for the type of failure event (between the two notches versus across the sample width) were compared to the experimental results, the two were found to be strongly covariant by Fisher's exact test ($p < 0.05$) for both the aligned and isotropic gels with no fitting parameters. Although the double-notch system is idealized, and the collagen gel system is simpler than a true tissue, it presents a simple model system for studying failure of anisotropic tissues in a controlled setting. The success of the computational model suggests that the multiscale approach, in which the structural complexity is incorporated via changes in the model networks rather than via changes to a constitutive equation, has the potential to predict tissue failure under a wide range of conditions. [DOI: 10.1115/1.4031570]

Introduction

For decades, much of soft-tissue biomechanics research has focused on prefailure behavior (i.e., behavior in the range of strains over which the mechanical response is reversible, as described in the essential texts of Fung [1] and Humphrey and Delange [2], as well as recent papers such as Refs. [3–5]). Prefailure studies have the advantage of not necessarily destroying the sample, and modeling prefailure behavior is attractive because the prefailure behavior of a complex tissue can often be interpreted in terms of a summation of contributions from its various structural components. Failure, in contrast, presents a challenge to the experimenter in that the failure test destroys the sample and is prone to greater variability; the modeler is also challenged because failure occurs locally rather than globally, and the failure of one component can cause load transfer to another.

Failure events, however, are biomechanically critical in settings ranging from the repair of tendon injuries [6] and dermal wounds [7] to the rupture of aneurysms [8], so it is imperative that we pursue biomechanical modeling of failure as well as prefailure behavior. In the area of cardiovascular mechanics, Gasser and Holzapfel [9,10] have used a cohesive-zone model for vessel dissection and plaque failure, and Pei et al. [11,12] have recently applied classical crack growth analysis to a model of an atheromatous plaque, but models of tissue failure remain rare, in particular when the failure site is not predetermined.

It is also well recognized that crack propagation is an important factor in tissue damage and is highly influenced by the structure of a tissue. Dissection of an ascending thoracic aortic aneurysm, for example, tends to run along the z - θ plane, delaminating the

vessel wall between elastic layers [13,14], and the characteristic failure of the glomerular basement membrane in Alport syndrome has a similar delaminating quality [15]. The angle-ply structure of the annulus fibrosus is known to provide increased strength [16], and tendons and ligaments are extremely effective at resisting propagation of transverse tears [17]. The toughness of fiber composites is well established in nonbiological contexts, but translation of fiber-composite concepts to the failure of tissues containing a complex network of interacting fibers is by no means trivial.

We [18,19] and others [20–22] have previously attempted to model tissue failure using a microstructural representation of the tissue's architecture within a multiscale context or by modeling a relatively small piece of tissue in terms of a set of connected elements. This approach is attractive because it allows the failure problem to be reduced from a three-dimensional, spatially varying tissue problem to a one-dimensional fiber problem. Also, because no special elements are required, the approach allows one to simulate the entire tissue without a priori knowledge of where the crack will occur. Such models are computationally demanding but can provide important insights into the failure process.

The fibroblast-populated collagen gel is a long-established model tissue [23–25], providing a highly reproducible system that can be

- fabricated in the laboratory;
- manipulated mechanically [26–28] or magnetically [29,30] to generate alignment of the constituent collagen fibers;
- tested under various loading configurations [31,32]; and
- imaged via quantitative polarized-light imaging [33,34].

We have previously shown [18] that the stress-strain curve, including the point of failure, of a collagen gel under uniaxial load can be generated accurately using a multiscale model, and that the same model can be regressed to data from dogbones and

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Fig. 1 Typical samples: (a) alignment plot of a nominally isotropic sample cut from a cruciform mold. The direction of the vectors indicates the primary direction of alignment as measured by quantitative polarized-light microscopy [33], and the length of the vector indicates the degree of alignment. Although there is some alignment, particularly near the edges (near the arms of the original cruciform), the sample is close to isotropic. (b) Alignment plot for an anisotropic sample from a uniaxial (dogbone) mold. Very strong alignment is seen throughout the sample. (c) Photograph of a sample under initial tension showing the notch tip-to-edge and notch tip-to-tip distances, which were used to characterize samples.

then used to predict the results from notched dogbones. The current work used a double-notched collagen gel dogbone model to characterize the failure behavior of isotropic and anisotropic gels.

Methods

Experiment. Cell-driven compaction of mechanically constrained collagen gels was used to induce alignment according to the following protocol. Uniaxial (i.e., dogbone shaped) and biaxial (cruciform) molds into which the collagen gels would be cast were cut from a sheet of Teflon and sealed in a sterile Petri dish with vacuum grease. Uniaxial molds anchored the collagen gel at the dogbone ends, and biaxial molds anchored the gel at all four arms. Collagen solutions were made as described previously [35], containing 2.0 mg/ml collagen in a solution of high-glucose DMEM with 10% fetal bovine serum (Hyclone Laboratories, Logan, UT) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA). All samples used the same collagen concentration. A 500,000-cell/ml suspension of neonatal human dermal fibroblasts (Clonetics, Walkersville, MD) was added to provide enough cells to compact the gel and induce alignment. The cell-collagen mixture was pipetted into the mold and incubated for 30 min to ensure initial gelation. Media was then added to the dish, and the sample was incubated for 24 hrs at 37 °C, which allowed for rearrangement of the collagen fibers within the gel but was not long enough for significant synthesis or degradation of collagen (cf. Ref. [36]). During compaction, the arms (two for uniaxial and four for biaxial) were held fixed so as to induce strong anisotropy in the uniaxial samples and relative isotropy in the biaxial samples. Compacted uniaxial gels were transferred directly to the mechanical tests, and a dogbone-shaped section was cut from the center of the compacted cruciform biaxial gels. Figures 1(a) and 1(b) show

the alignment (as measured by quantitative polarized-light imaging) in the two different types of samples.

Samples were notched on both sides with surgical scissors. The depth and placement of the notches were varied for different samples so as to provide a range of conditions, and the distance between the two crack tips was recorded, along with the distance from a crack tip to the opposite edge of the dogbone; both distances were normalized by the sample's undeformed width (Fig. 1(c)). Each sample was mounted on two arms of an Instron biaxial testing system in uniaxial testing mode, using 5-N load cells and sandpaper-coated metal grips. The sample was then stretched at 0.4% per second, a rate slow enough that the system could be treated as quasi-steady but fast enough that the experiment could be completed without significant additional compaction. Video recording of the experiment was used to determine whether the sample failed by forming a connection between the two notches (called the BETWEEN case) or by the crack from one notch propagating across with throat of the dogbone (ACROSS), as shown schematically in Fig. 2. For each experiment, the result (BETWEEN or ACROSS) was recorded.

Multiscale Computational Model. Our multiscale model of collagen gel mechanics [37,38], adapted to simulate sample failure as well as prefailure behavior, has been described in detail previously. Briefly, a macroscopic-scale finite-element mesh is generated and is used as the platform for solving the continuous-averaged Cauchy stress balance. At each Gauss point of each element, however, the closed-form constitutive equation that might normally appear is replaced with a microscopic-scale network problem to represent the mechanics of the collagen network in a small volume surrounding the Gauss point. Each microscopic-scale network problem contained 300–500 fibers generated using

Fig. 2 Failure mechanisms: Samples failed either by a crack connecting the two notches (“between,” left side image) or by a crack propagating across the sample from one notch (“across,” right side image)

the Delaunay triangulation of a random point cloud. Fiber connections were treated as freely rotating joints. Details on the approach are given in Refs. [19] and [34].

A stretch was imposed on the sample by holding one end of the domain fixed and displacing all nodes on the other end. An initial guess of interior node displacements was made, and then, each microscopic problem was specified based on the guessed displacement field. After the fibers in each microscopic network had equilibrated, the average stress in the network was calculated and passed up to the macroscopic finite-element model, and nodal positions were updated via Newton iteration until the averaged stress balance was satisfied.

Prefailure fiber mechanical behavior was modeled, as in our previous studies (e.g., Refs. [19] and [34]), using an exponential force–stretch function (cf. Ref. [39]) for fiber force F_f

$$F_f = \frac{E_f A_f}{B} \left[\exp \left(B \frac{\lambda_f^2 - 1}{2} \right) - 1 \right]$$

where E_f is the modulus of a fiber in the small-strain limit, A_f is the fiber cross-sectional area, B is a nonlinearity parameter, and λ_f is the stretch ratio of a fiber. Damage was simulated by comparing the stretch in each fiber in each microproblem to a critical stretch ratio λ_{crit} . If a fiber exceeded the critical stretch, it was functionally removed from the network by a billionfold reduction in E_f . The damaged networks were then used for the next macroscopic displacement step, and the entire process repeated until failure. Modeling fiber failure in this way does not distinguish between fiber failure and failure of a crosslink between two collagen fibers. The values of the parameters were set to $E_f = 7.4$ MPa, $A_f = 7854$ nm² (100 nm diameter), $B = 0.25$, and $\lambda_{crit} = 1.42$, based on our previous work [18,19].

To model the notches, corresponding elements were removed from the idealized geometry for each simulation. Notches were made one element wide and one, two, or three elements deep in the sample. Notch position along the sample arm was varied, starting with notches close together and moving them apart. The failure of the sample was categorized as between the notches or across the sample as in the experiments (see Fig. 2).

Simulations were performed using networks with varying levels of fiber alignment, as characterized by the first component of the fiber orientation tensor, Ω_{11} ,

$$\Omega_{ij} = \frac{\sum_{\text{fibers } f} \ell_f n_i^f n_j^f}{\sum_{\text{fibers } f} \ell_f}$$

where ℓ_f is the length of fiber f , and n_i^f is the unit vector pointing in the direction of fiber f . The trace of Ω is unity by construction. For an isotropic network, $\Omega_{11} = \Omega_{22} = \Omega_{33} = 0.33$, and for a perfectly aligned network, $\Omega_{11} = 1.0$ while $\Omega_{22} = \Omega_{33} = 0$, but the maximum Ω_{11} value we considered was 0.62 because that value

Fig. 3 Model networks: Model networks were generated with different degrees of fiber alignment ranging from isotropic to strongly anisotropic. The values of Ω_{11} for the networks shown are 0.33, 0.4, 0.5, and 0.62 from left to right.

gave results that matched the highest degree of alignment attained in our experiments. In all cases, it was assumed that the sample was transverse isotropic, i.e., $\Omega_{22} = \Omega_{33} = (1 - \Omega_{11})/2$. Different representative networks for the different alignments studied are shown in Fig. 3.

Comparison Between Model and Experiment. For each set of networks, a plot of tip-to-edge distance versus tip-to-tip distance was constructed, and a line of separation was drawn between the between and across points to minimize the p -value for Fisher’s exact test on the two-by-two system of (BETWEEN, ACROSS; above line, below line). The null hypothesis for this test was thus that a BETWEEN or ACROSS failure event in an experiment was equally likely on either side of the calculated dividing line, and rejection of this hypothesis (i.e., a significantly low p -value) indicated that a sample notched with conditions that place it above (below) the line is more likely to have a BETWEEN (ACROSS) failure event.

Results

Model. Simulated force–stretch data were similar to those seen in our earlier single-notch simulations [18,19], with a toe region and then a rounded force peak and sample failure. A repeatability study on six realizations of the microstructure for the same problem yielded variation (standard deviation divided by the mean) of 4.1% for failure force and 3.7% for failure (grip) strain, consistent with our previous findings [40,41], and no change in the BETWEEN/ACROSS results. The effect of alignment on the force trace was consistent with our previous study [19] and will not be discussed in the current work. Rather, we focus on the location of the tissue failure. As shown in Fig. 4, when the initial notches were short and near each other, the crack tended to connect the two notches and form a BETWEEN failure case. In contrast, when the initial notches were longer and farther apart, an ACROSS failure resulted; in the ACROSS case, both cracks tended to grow but at slightly different rates, so in the end one crack reached the opposite edge before the other did.

For each given alignment, a series of simulations were done with different notch depth and separation distance (Fig. 5). The separation line between BETWEEN and ACROSS for anisotropic networks is shifted to the right from that for the isotropic networks, indicating that the BETWEEN failure becomes more likely as the network becomes more strongly aligned. For example, for a normalized tip-to-tip distance of 0.8 and a normalized tip-to-edge distance of 0.7, the simulated isotropic sample is in the ACROSS region, but the simulated aligned sample is in the BETWEEN region.

Experiment. The force traces for the experiments were as expected, with a toe region, steady increase in force, and drop to zero force as the sample failed (e.g., Fig. 6). The smooth curve at failure was consistent with our previous study [18], in which we

Fig. 4 Failure behavior of simulated gels: Depending on the size and separation of the notches, simulated gels failed either by forming a single crack running BETWEEN the two notches (left-hand column) or by forming two separate cracks that ran ACROSS the sample (right-hand column)

Fig. 5 Model results: Model predictions for BETWEEN (triangles) and ACROSS (squares) failure are given for isotropic ($\Omega = 0.33$, solid symbols) and anisotropic ($\Omega = 0.62$, solid symbols) samples. The dotted line separates BETWEEN from ACROSS conditions for the isotropic simulations, and the dashed line separates BETWEEN from ACROSS conditions for the anisotropic simulations. In both cases, samples with the notches deeper into the tissue (lower down on the plot) and samples with the notches farther apart (to the right on the plot) tended to fail ACROSS the sample, whereas those with shorter and closer notches tended to fail BETWEEN the notches. The dashed line has a slightly higher slope and is shifted considerably to the right when compared to the dotted line, showing that the aligned model is more likely to fail between the notches.

observed smoother failure curves during crack propagation across the sample than in un-notched samples. Averaging over all samples (without any accounting for differences in geometry), we found that the samples with the ACROSS failures occurred at higher grip strain than the BETWEEN failures (28.3% versus 21.8%, $p = 0.011$ by a two-tailed t -test); the BETWEEN failures occurred at a slightly but not significantly lower force (92.8 ± 49.3 mN versus 100.7 ± 22.0 mN, $p = 0.56$).

Figure 7 shows the BETWEEN versus ACROSS results for all experiments, with the isotropic samples in Fig. 7(a) and the anisotropic samples in Fig. 7(b). For the isotropic case, the model-predicted separation line divides the data quite well, with 14 of the 16 results being as predicted by the model. Although Fisher's exact test p -value for the two-by-two system (BETWEEN-model, ACROSS-model; BETWEEN-experiment, ACROSS-experiment)

Fig. 6 Force trace: Typical force trace for isotropic (dashed line) and anisotropic (solid line) samples. As expected based on Ref. [18], there is a discernable toe region, a linear region, and a relatively flat peak region, and finally, a drop to zero force when the sample failed. The quantitative features of the force trace depended on the alignment of the gel and on the size and positions of the notches.

is not meaningful in the usual sense, it provides a measure of the quality of the prediction. For Fig. 7(a), the p -value is 0.0087, meaning that the division suggested by the model correlates very strongly with that observed experimentally. As expected, in the anisotropic samples, the separation line shifted to the right, indicating a greater likelihood of the BETWEEN result. The model prediction for the $\Omega = 0.62$ case (dashed line) gives a p -value of 0.028. Because of the uncertainty in estimating the fiber alignment, we also show the $\Omega = 0.5$ model results (gray line), which are shifted slightly to the left; the p -value for the $\Omega = 0.5$ case is 0.091. Given that the model results of Fig. 7 were obtained with no fitting parameters, we conclude that the model is able to capture the shift in the failure process for a collagen gel as the sample becomes more strongly aligned.

Discussion

It is well known that tissues tend to fail in specific ways that relate to their fiber orientation [13–17]. In this work, we demonstrated that a multiscale model with a simplified fiber-based failure algorithm could predict the crack propagation pattern in a double-notched sample, and that the model predictions were in agreement with experimental observations on collagen gels. To our knowledge, although there have been other double-notch systems used in tests of synthetic materials [42,43], none has been applied to tissue or has used the offset-notch geometry of the current study. The approach described herein offers an easy system in which to examine the differences in crack propagation with direction, with the ACROSS/BETWEEN dividing line appearing to be quite well defined over many experiments, and with much smaller error in the estimated quantities (slope and intercept of the dividing line) than in, for example, the grip strain and tensile force at failure in a dogbone-shaped gel [19]. The double-notch model is a potentially valuable tool to characterize the failure of an anisotropic tissue, contrasting with standard crack propagation tests by allowing the crack to propagate in one of two different cardinal directions. It would be informative, for example, to study how anisotropic synthetic gels [44,45] or tissues [14,46–48] behave under double-notch testing, and also to study theoretically whether different network architectures lead to different double-notch test results.

The theoretical model used in this study remains an extreme simplification of the actual failure process. There was no fatigue in the sample, all fibers had identical prefailure and failure properties, and the loading was always in the preferred fiber direction. The work of Shen et al. [49,50], for example, suggests that collagen fibrils can exhibit a wide range of failure stretches, which

Fig. 7 Between versus across failure in experiments: In both plots, the triangles indicate failure between the notches, and the squares indicate failure across the sample (same symbols as in Fig. 5). Each symbol corresponds to a single, independent experiment. (a) For isotropic samples, the between/across results are largely consistent with the model prediction (dotted line repeated from Fig. 5(a)). (b) For anisotropic samples, as expected, the division between BETWEEN and ACROSS is shifted to the right. The dashed line is the $\Omega = 0.62$ simulation result from Fig. 5(b), and the solid gray line is the simulation result for a less strongly aligned case ($\Omega = 0.5$).

might be important in future studies. The collagen gel system is also simple in that it contains only one mechanically significant component. In a real tissue, there are other structural components (e.g., elastin and proteoglycans) as well as cells contributing to the behavior, and the loading conditions may be extremely complex, especially if the tissue has an irregular geometry.

The model also had no accounting for the role of cells in the failure process. The cell density in the gels, even after compaction, was relatively low, so we would not expect a percolation of the sample by cells (as discussed in Ref. [51]) but rather a situation in which cells were largely isolated and surrounded by a dense region of compacted collagen [52–54]. How these cells affect the failure behavior of the sample is not clear. One must conclude from the success of the model, which was regressed to data from acellular gels, that the effect of the cells in this system was small, at least with respect to the BETWEEN/ACROSS divider, but there is clear need for further study on the collective contribution of cells and matrix to tissue failure. Although we found cell-driven compaction to be simple, low-cost, and effective, if one were concerned about the role of the cells, it would be possible to produce an aligned acellular collagen gel by, for example, gelation in a strong magnetic field [29,30] or electrospinning collagen fibers [55].

The current work also produced two results that merit further discussion. First, this study, like our previous theory-only study on failure of aligned gels [19], emphasizes the importance of the fundamental principle that a material fails when its stress exceeds its strength, and that both the stress and the strength can vary with position and direction. Changing the alignment of the fibers in a double-notched collagen gel changed the way in which the gel failed because it shifted strength from one direction to another, leading to different responses to the same applied load. As finite-element tools get better and better at predicting the stress field in a tissue, predicting failure will require better constitutive models that account for anisotropy in the failure criteria, as is already common in simulating the prefailure behavior (e.g., Refs. [56–58]).

A second consideration, especially if one is interested in modeling tissue failure, is that the current work employed the simplest possible microscale model of failure—each fiber was one-dimensional and failed at the same critical stretch, and no distinction was made between failure within a fiber or at the junction between two fibers—but was able to capture larger-scale behavior

via changing the organization of the simple constituent elements. That approach—a simple failure model based on a complex microstructural model—could be important in understanding how changes in tissue structure (e.g., due to remodeling) lead to changes in anisotropic tissue strength.

Our success in this regard has both positive and negative implications. It is heartening to think that complex phenomena can be described with such simple underlying elements if arranged properly, but it is also daunting to recognize the importance of describing structure accurately if one hopes to make accurate predictions. Fortunately, advances in structural analysis of tissue [59–63] and in direct creation of structural models from image data [64] are steadily improving our ability to obtain and use the necessary data.

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