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THE MECHANICS OF NETWORKED, TYPE II COLLAGEN FIBERS FROM CARTILAGE

Phoebe Szarek (1), David M. Pierce (1,2)

(1) Department of Biomedical Engineering, University of Connecticut, Storrs, CT, USA
(2) Department of Mechanical Engineering, University of Connecticut, Storrs, CT, USA

INTRODUCTION

Collagen, a key load-bearing molecule essential to the function of most soft tissues and load-bearing organs, is the most abundant protein in the mammalian world [1]. Understanding the mechanics of collagen is thus critical to understanding the biomechanics of soft tissues and organs. Type I collagen is the most abundant collagen in the human body (e.g. in dermis, tendons, and arteries), so researchers have extensively characterized the mechanics of type I collagen [2]. Type II collagen forms networks and is the second most abundant type in the human body (e.g. in hyaline cartilage and intervertebral discs), but *no data exist* on the tensile mechanical response of individual type II collagen fibers. Experimental testing of individual fibers of type II collagen poses many difficulties due to their small size (Ø 20-200 nm) and due to the complexity of *in vitro* fibrillogenesis [3,4].

We aim to quantify the mechanics of individual type II collagen fibers via inverse analyses of fiber networks with measurable bulk responses and fiber arrangements. Specifically, we aim to 1) identify an appropriate constitutive model for the stress-strain response of type II collagen fibers, 2) identify an appropriate failure model (criteria) for individual type II collagen fibers, and 3) determine the parameters for these models by leveraging our diverse experimental data.

METHODS

Specimen Preparation. Following established protocol, we extracted 10×20 mm full-thickness specimens from bovine patella-femoral grooves (< 36 m old) [5]. We microtomed 140 or 160 µm slices from the superficial zones (SZ) and punched out dumbbell-shaped specimens (gauge width = 3 mm, length = 4 mm). We incubated these in phosphate buffered saline (PBS) with 0.5 mg/mL trypsin for 18 hrs at 37 °C to remove >95% of the proteoglycan (PG), then rinsed.

Uniaxial Tension Testing. We performed uniaxial tension tests using a custom microtensile device (20 N capacity with 0.3 mN resolution, 12.5 mm range with 2.5 µm resolution) at a displacement rate of 300 or 600 µm/min until failure in PBS at room temperature [5].

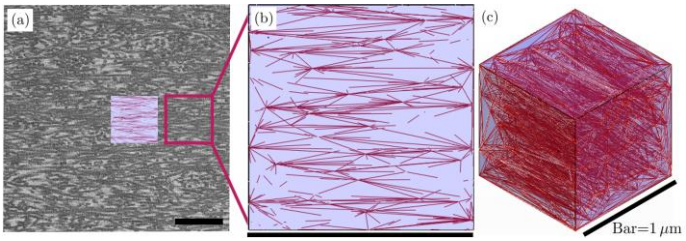

Figure 1. Representative Statistically-Equivalent Representative Volume Element (SERVE) for modeling networks of collagen fibers. a) Transmission electron microscope image of collagen within the superficial zone of human cartilage. b) Cross section of a fiber-network SERVE model. c) RVE with statistically equivalent orientation, dispersion, and volume fraction of collagen fibers [7].

Statistically-Equivalent Representative Volume Elements. We modeled a 1 µm^3 cube, i.e. a statistically-equivalent representative volume element (SERVE), at the center of the gauge region of the tensile specimen using FEBio (University of Utah) [6]. We used data on the orientation, diameter, and volume fraction of type II collagen fibers within the SZ (measured via Transmission Electron Microscope images [7]) to create statistically-equivalent networks of fibers within multiple SERVEs (Fig. 1). We modeled fibers as springs (selecting from five different constitutive models) and we modeled the PG-depleted interfibrillar space using a biphasic neo-Hookean model ($G = E_{\mathrm{iso}}/3$).

Constitutive Models. We used five different constitutive models for individual fibers: 1) linear, 2) exponential [8,9], 3) bilinear (i.e. toe and linear regions [10]), 4) exponential-linear, and 5) linear-exponential. We tested each of these models by fitting the stress-strain responses of networked collagen to data using inverse finite element modeling. Leveraging each of these constitutive models we fit the responses of the each SERVE to measured data. We assessed the model fits using both the coefficient of determination R^2 and number of parameters required.

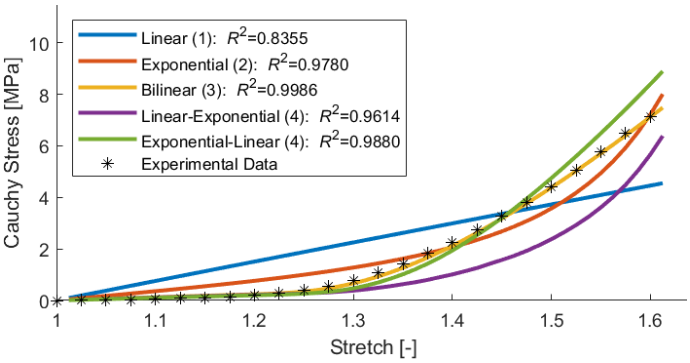

Figure 2. Representative best fits of five constitutive models. The bilinear model provided the best fit to the experimental data even compared against models with more parameters (in parentheses).

Failure Models. To model failure of individual type II collagen fibers we extended the constitutive models to include failure, and tested two failure criteria: 1) critical stress and 2) critical stretch. In the first failure criterion, e.g. if the stress exceeds a critical value the fiber is removed from the network. We rerun each time step of the simulation up to ten times to redistribute the total load, remove fibers, and stabilize the results. We assessed both failure models within SERVEs to establish which better predicted the network responses measured experimentally.

Data Analyses. We performed inverse fitting of the experimental data (n = 10) using the parameter optimization module in FEBio. We fit the first 10% of the experimental data to establish the stiffness (shear modulus) of the isotropic matrix. We then fit the stress-strain data up to bulk softening (i.e. decrease in slope) using the different SERVEs with fibers (n = 3) to fit the parameters of the individual fiber response. Finally, we fit the failure criteria using the complete data to rupture.

Statistical Analyses. Grouping all the data we used t-tests to discard SERVEs with responses significantly different from all others. We used two-way ANOVAs to assess the variance due to both experiments and network models (SERVEs). We used p = 0.05 as our level of significance and reported median and interquartile range (M + IQR).

RESULTS

Constitutive Models. Figure 2 shows a representative network response and the best-fits of the models. We found that a bilinear model

$$\sigma = \begin{cases} E_1(\lambda - 1) & \text{if } \lambda < \lambda_t \\ E_2(\lambda - \lambda_t) + E_1(\lambda_t - 1) & \text{if } \lambda \geq \lambda_t \end{cases}, \qquad (1)$$

where $E_1 < E_2$ are the slopes of the bilinear fiber model which transition at the stretch λ_t, provided the simplest and best-fit for the response of single fibers. Table 1 shows the resulting parameters after fitting all of the experimental data with the bilinear model. We found that responses from the SERVEs were statistically equivalent and had no effect on the model parameters. The fiber parameter most affected by the SERVEs, albeit not significantly, was the transition stretch.

Table 1. Bi-linear Model Parameters for Type II Collagen Fibers.

	E_{iso} [MPa]	E_1 [MPa]	E_2 [MPa]	λ_t [-]
M	1.015	0.9526	23.27	1.138
IQR	0.8100	0.9558	4.919	0.0649

Failure Models. Figure 3 shows the effects of adding mechanical criteria to model individual fiber failures within the networked collagen. Failure based on critical fiber stress more accurately captured the bulk network responses measured experimentally versus critical fiber stretch.

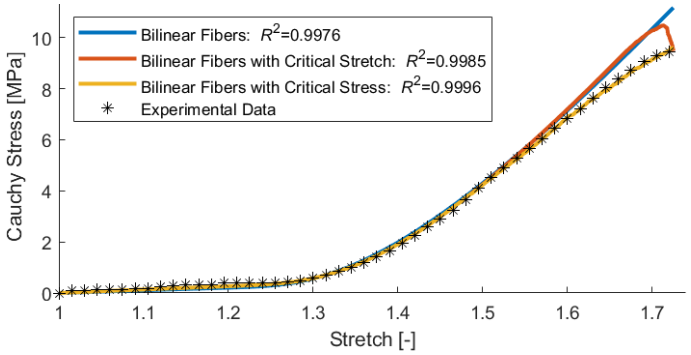

Figure 3. Representative best fits including fiber failure. Maximum fiber stress more accurately captured the bulk network response measured experimentally versus maximum fiber stretch.

DISCUSSION

We propose a new fitted model for networked, type II collagen fibers within cartilage, and potentially within other soft tissues containing type II collagen. The bilinear model for a single fiber is comparable to the toe and linear regions seen in highly aligned collagenous structures. We model each fiber with zero-order continuity, but obtain a first-order continuous network response due to progressive recruitment of fibers. While we tested and modeled tension to rupture, physiological stretches are often less extreme, possibly explaining the usability of the common exponential model. Our model parameters also indicate that type II collagen may not be as stiff as type I collagen, cf. [2]; differences in testing methods may also explain the discrepancies.

Introducing fiber failure into our constitutive model allowed fitting of the complete experimental data and a better estimation of fiber and network responses at large strains. In our experimental data, we identified cases where networks abruptly failed making fitting of individual fiber failures difficult. Fiber failure with a stress-based criterion better represented network failure with softening but a stretch-based failure may better represent catastrophic network failure.

Limitations of this work include difficulty in decoupling the isotropic stiffness and the low-strain stiffness of the collagen fibers. Unfortunately, we are unable to run simulations with very low stiffness PG (e.g. the networks collapse) or past complete rupture (indeterminate solution). While it is primarily type II, collagen within cartilage is also heterotypic with small quantities of other types of collagen.

We introduce a new mechanical model for type II collagen fibers, a key load-bearing molecule in the human body. Our findings may serve to improve current modeling approaches and introduce opportunities for multiscale modeling. Including a failure criterion for individual fibers results in a better fit to experimental (data for networks tested to failure), a more accurate fiber response for physiological models, and the possibility to model softening under cyclic loading.

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