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A Model for the Stretch-Mediated Enzymatic Degradation of Silk Fibers

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Abstract

To restore physiological function through regenerative medicine, biomaterials introduced into the body must degrade at a rate that matches new tissue formation. For effective therapies, it is essential that we understand the interaction between physiological factors, such as routine mechanical loading specific to sites of implantation, and the resultant rate of material degradation. These relationships are poorly characterized at this time. We hypothesize that mechanical forces alter the rates of remodeling of biomaterials, and this impact is modulated by the concentration of enzymes and the duration of the mechanical loads encountered *in situ*. To test this hypothesis we subjected silk fibroin fibers to repeated cyclic loading in the presence of enzymatic degradation (either α -chymotrypsin or Protease XIV) and recorded the stress-strain response. Data were collected daily for a duration of 2 weeks and compared to the control cases of stretched fibers in the presence of phosphate buffered saline or non-stretched samples in the presence of enzyme alone. We observed that incubation with proteases in the absence of mechanical loads causes a reduction of the ultimate tensile strength but no change in stiffness. However, cyclic loading caused the accumulation of residual strain and softening in the material properties. We utilize these data to formulate a mathematical model to account for residual strain and reduction of mechanical properties during silk fiber degradation. Numerical predictions are in fair agreement with experimental data. The improved understanding of the degradation phenomenon will be significant in many clinical repair cases and may be synergistic to decrease silk's mechanical properties after *in vivo* implantation.

1. Introduction

The study of biomaterial degradation represents an important though often overlooked topic within the field of tissue engineering. The ability to predict and control the rate of degradation of tissue scaffolds is critical for the design of load-bearing tissue replacements. This becomes apparent *in vitro*, where slow degradation of the supportive scaffold is necessary to accommodate cellular infiltration and new tissue formation mediated by cells. Likewise, *in vivo*, degradation facilitates the body's integration of foreign biomaterials into the host tissue. The consideration of degradation processes is of particular clinical importance when replacement tissues are to serve mechanical functions. In such instances, clinical complications

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can arise when degradation outpaces the rate at which replacement tissue can form and assume load bearing responsibilities. As such, degradability must be predictable so that tissue regeneration occurs at a rate that parallels the degradation of the scaffold, allowing transition of mechanical loads and biological functions during turnover. Without this correspondence, full regeneration could be undermined by one of two clinical outcomes: (i) degradation occurs too rapidly resulting in loss of mechanical function and tissue failure, as found in early studies of ligament repair with collagens (Chvapil et al., 1993); (ii) degradation occurs too slowly, resulting in barriers to transport and cell infiltration, sites of necrosis, stress shielding, and ultimately a lack of autogenous tissue formation (Woods, 1985). Despite these design challenges, recent reports have confirmed that slow scaffold degradation can be matched by gradual new tissue formation in order to sustain function (Horan et al., 2009a, b). This method of *functional tissue engineering* can be vital for successful clinical outcomes using degradable materials (Butler et al., 2000).

Investigators have increasingly been drawn to silk-based biomaterials because they offer inherently slow rates of degradation as well as impressive mechanical properties, a rare and valuable combination. Silk-based biomaterials are available in a variety of forms, however, the most widely used in clinical applications is the textile form (Altman et al., 2003). For example, silks have been used as sutures for decades under FDA approval, and are less inflammatory than stiff and degradable polyesters such as PLGA and PLLA (Altman et al., 2003; Panilaitis et al., 2003). To date, textile silks have been engineered for applications such as degradable Anterior Cruciate Ligament (ACL) and hernia repair prosthetics (Altman et al., 2008; Horan et al., 2009a, b) as well as functional tissue engineering scaffolds of the ACL and annulus fibrosus of the intervertebral disc (Kandel et al., 2008; Altman et al., 2002; Fan et al., 2008, 2009). In order to effectively utilize silk-based biomaterials, it is important to anticipate their slow but still significant rates of degradation *in vivo*. For example, studies on hernia repair *in vivo* reveal rapid decreases in mechanical strength ($\approx 50\%$) within a 2 – 3 week time frame (Horan et al., 2009a). As a result, the authors chose to overdesign the degradable construct at 3 – 4 times the normal physiological strength requirements, showing that healthy modes of ECM deposition will reinforce and eventually supplant the slowly weakening scaffold architecture.

Changes in the mechanical properties are not apparent in actively stretched or static tissue culture studies performed *in vitro* (Altman et al., 2002); however, substantial softening of the supportive scaffold can be induced when they are exposed to natural proteases (Horan et al., 2005). This discrepancy between *in vitro* and *in vivo* behaviors strongly suggests that the body's response to the implant pose significant challenges to silk's resiliency, accelerating turnover rates. To date, the combined impact of two dynamic factors - load and physiological enzymatic attack - have not been replicated through *in vitro* simulation, despite their significant impact on scaffold durability. We suspect this lack of understanding is due to many technical challenges and complexity related to mimicking the physiological conditions of the native implant site. Unfortunately, in the absence of accurate *in vitro* paradigms, the field has become reliant on expensive, time-consuming studies of animal models. If we could refine *in vitro* modeling of silk and other biomaterial degradation by accounting for enzymatic processes as well as mechanical loads, we could more accurately approximate the long-term durability *in vivo*.

In our experimental work, we sought to address the practical challenges involved in recapitulating the same coupled processes (load and environment) *in vitro* that impact silk degradation *in vivo*. To accomplish this aim, we utilized an experimental apparatus for measuring simultaneous loading/unloading responses in the presence of various enzymes at physiologically-relevant conditions. We hypothesized that mechanical loading indeed alters the rates and nature of degradation of biomaterials, but that this process is dually driven by the types of enzymes encountered *in situ*. We further hypothesized that available constitutive

models could accurately capture the evolving mechanical behavior of these systems. To test these hypotheses, we obtained experimental data on the degradation process of silk during cyclic tensile loading and various enzyme treatments. Following data collection, we adapted an existing mathematical framework to account for evolving material properties (Demirkoparan et al., 2009). The ability of the model to reproduce our experimental data is then used to define the relevance of the approach towards predicting end-use functionality of silk-based biomaterials. More broadly, development and refinement of these models will help to formalize the “design rules” for tissue engineers, offering more accurate predictions on material performance in the clinic as they apply to degradable biomaterials used in load-bearing applications.

2. Experimental Methods and Materials

A series of experiments were performed to improve our understanding of the degradation process of silk fibers when exposed to combined enzymatic factors and mechanical loads. To quantify the type and magnitude of degradation, we first establish baseline data by exposing fibers to Phosphate Buffered Saline (PBS) and perform monotonic extension as well as cyclic loading-unloading tests. Subsequently, fibers were exposed to the enzymes Protease XIV and α -chymotrypsin and subject to the same mechanical loading sequence. In all cases, different times of exposure were used and the corresponding data compared.

2.1. Materials preparation

Raw silk yarns (imported by Rudolph-Desco, Inc.) were extracted for 60 minutes using an aqueous solution containing 0.02 M Na₂CO₃ (Sigma-Aldrich) and 0.3 % Ivory Soap detergent as previously described (Altman et al., 2002). Prior to extraction, yarns were plied to 4 ends and 4 of these bundles were lightly twisted to form a 16-fiber construct using a CalvaniTM Fancy Jet model 6/SP Ring Twister (Milan, Italy).

Enzyme types and concentrations were selected based on their physiological relevance, known activity levels from previous studies, and known ability to significantly degrade mechanical properties within a two-week time frame (Horan et al., 2009a, b). Solutions were prepared as described in (Horan et al., 2005). Briefly, both α -chymotrypsin (Sigma Product# C3142) and Protease XIV (Sigma Product# P5147) were resuspended in Dulbecco's Phosphate Buffered Saline (PBS, Invitrogen) at 1 mg/mL concentration (64 U/mL and 4.9 U/mL activity, respectively). Due to the large volume of enzyme solution used (10 mL = 10 mg enzyme) relative to the substrate (1 yarn $\approx$ 3.25 mg), the solutions were changed every third day rather than daily. Others have reported enzyme activity of > 50% after two weeks (Arai et al., 2004).

2.2. Monotonic loading

We applied a monotonically increasing stretch to determine the quasi-static stress-strain behavior and the ultimate load carrying capacity of silk fibers (Horan et al., 2005) for details. Briefly, fiber samples (n=4) were hydrated in either PBS or 1 mg/mL Protease XIV for periods of 0, 3, 7, 9, 12 and 14 days, with solution replaced every third day. Before being mounted to an InstronTM load frame, the samples were washed thoroughly with PBS. All uniaxial extension tests were performed in two steps. First, the fibers were stretched very slowly until a preload of 20 mN was reached. Afterwards, a monotonically increasing stretch was applied with a constant strain rate of 0.001 sec⁻¹ until failure. Applied load and strain (based on crosshead displacement) were recorded at constant intervals of 0.1 seconds and reported as nominal stress versus strain. The stress is calculated as the applied load over the original cross-sectional area. A “silk yarn” is a 16-fiber construct, where each fiber consists of 5 filaments (the extruded form of material from the silkworm). We assume that each filament has a circular cross-

sectional area and diameter of 9 μm , which has been measured elsewhere by scanning electron microscopy (Horan et al., 2005). Thus, for a 16-fiber yarn, composed of 80 filaments, the final cross sectional area of the entire yarn is estimated at 0.0051 mm^2 . All yarn samples for this study were taken from the same reel.

2.3. Cyclic loading

To assess the added influence of mechanical loads on the degradation of silk fibers, pairs of fibers were symmetrically mounted in a bioreactor loading device and repeatedly stretched. The total duration of each experiment was 14 days, each using a different enzymatic treatments including Protease XIV, α -chymotrypsin and PBS as a base line control. The custom built bioreactor is equipped with specially-designed grips featuring a pivoting gripping head that allows for uniform stress application to each yarn bundle while submerging the pair of fibers completely in the solution. A detailed description of the bioreactor is outside the scope of this paper and will be given in a forthcoming publication. For each condition, the fibers were first incubated in a chamber containing PBS, and stretched at a slow constant rate until a 20 mN tare load was reached. Next, a 15-cycle preconditioning with constant strain rate of $\pm 0.001 \text{ sec}^{-1}$ and maximum strain of 0.025 was performed on each specimen to determine the baseline mechanical properties.

Experimental evidence has shown that preconditioning of polymer based materials is necessary to obtain repeatable stress-deformation responses (Dorfmann and Ogden, 2004; Dorfmann et al., 2007; Lin et al., 2009). In particular, silk fibers show large differences in the stresses under loading and unloading during the first cycle in periodic tests with a fixed strain amplitude. Also, accumulation of residual strain occurs, again the major part during the first loading-unloading cycle (Perez-Rigueiro et al., 2000). After multiple cycles, the stress- deformation response is essentially repeatable and additional stress softening and residual strain generated are negligible. To characterize enzymatic degradation, we are interested only in the repeatable material response. Thus, data showing progression of stress softening and accumulation of plastic strain in the preconditioning stage at the beginning of the experiment are omitted for clarity.

We denote the mechanical response corresponding to the 15th loading-unloading cycle as the *virgin* (repeatable) mechanical behavior of the material, and labeled “Day 0”. Thereafter, except for the control non-enzymatic case, the PBS solution was replaced by either Protease XIV or α -chymotrypsin. The material was given a 24-hour time frame to adjust to the new environment. On each subsequent day, the material was subjected to 60 loading-unloading cycles with a constant rate of $\pm 0.001 \text{ sec}^{-1}$ with maximum strain of 0.025. During cyclic loading, the applied load was recorded at intervals of 0.1 sec. The data corresponding to the 30th cycle (the half point of the daily stretch regime) were used as the characteristic stress-strain behavior for this day. Destructive mechanical testing, as described in Section 2.2, was performed on day 14 to each sample after being removed from the bioreactor.

3. Experimental Results

3.1. Monotonic loading

Before mechanical testing, individual silk fibers were incubated at 37 °C in the presence of Protease XIV for durations of 0, 3, 7 and 14 days. During the incubation time, the protease solution was replaced every 3 days. To evaluate the effect of exposure time on the mechanical properties, single fibers were subjected to monotonic extension tests up to failure. The corresponding results are shown in Figure 1 as nominal stress versus applied stretch. Interestingly, the protease treatment in the absence of mechanical stress does not alter the stiffness of the material but substantially reduces the ultimate tensile strength and elongation

at failure with time of exposure, similar to results shown in (Horan et al., 2005). The stress-deformation response of the fiber incubated in PBS for 14 days is shown in Figure 1 as a dotted line and used as the baseline response to quantify the enzyme induced fiber degradation.

3.2. Cyclic loading

In the previous paragraph we found that exposure to Protease XIV reduces the ultimate load carrying capacity in silk fibers but leaves the stiffness unchanged, see Figure 1. We now show that the addition of mechanical loads during incubation significantly alters the stress-strain response over time. In Subsection 2.3 we described the loading protocol for cyclic loading. Recall that a pair of fibers is mounted inside the bioreactor and subjected to 60 loading-unloading cycles each day for the duration of the experiment. In particular, we consider a pair of fibers exposed to PBS, a pair exposed to Protease XIV and an addition pair to the enzyme α -chymotrypsin. Data are stored for each cycle on each day of the experiment. It is not necessary to show all the data to quantify the degradation process.

Figure 2 show the 30th loading-unloading cycle of silk fibers exposed to PBS on days 0, 5, 7, 9, 12 and 14. Note that overlapping graphs are *omitted* for clarity. Like many other biological soft tissues, silk fibers stiffen exponentially for increasing extension due to fiber recruitment and alignment along the loading direction. These data are used as control case since no degradation occurs and no permanent set accumulates during the 2 week testing period.

The material behavior shows significant softening and permanent set when exposed to Protease XIV and a daily loading-unloading sequence. Data in Figure 3 again show that the increase in stress with elongation is exponential for the range of deformations considered. There are also differences in the stresses corresponding to the same strain level under loading and unloading indicating energy dissipation. The difference is largest at the beginning of the treatment and becomes rather small after about 10 days of exposure. Residual strains are evident after 2 days of exposure, which increase throughout the duration of the test.

In the next experiment we replace Protease XIV with α -chymotrypsin and again evaluate the degradation during 14 days of exposure and daily loading-unloading cycles, see Figure 4. The data correspond to the 30th loading-unloading cycle on days 0, 5, 7, 9, 12, and 14. For clarity, overlapping graphs are again not included. Results clearly show that the amount of degradation of silk fibers depends on the particular kind of enzyme, Protease XIV being the most aggressive. Figure 4 shows stress softening and permanent set, but to a much lesser extent compared to data in Figure 3.

4. Modeling the Mechanical Response

Repeated loading-unloading of virgin silk fibers with maximum deformation below a critical value has a negligible impact on material degradation, residual strains and stress softening. However, in the presence of enzymes combined with periodic loading-unloading we observe significant material degradation. The enzyme-induced degradation is a complex phenomenon and available constitutive models are limited. A suitable framework was first proposed by Rajagopal and Wineman (1992). Their idea is based on deformation induced microstructural changes of the virgin polymeric network, such as a continuous breakage of cross links and the subsequent formation of a second different network. The new network is assembled in the deformed configuration, is stress free at the instant of formation and generates a permanent set in the material once the applied load is removed. The transient-network theory was also the starting point to develop a constitutive theory to describe degradation of biopolymers (Soares et al., 2008, 2010). They assumed that the material properties change with deformation and time and that a scalar field can be defined to account for the degradation. The transient-network theory was recently generalized to treat transversely isotropic materials (Demirkoparan et al.,

2009). One of their assumptions is that microstructural changes are restricted to the fibrous network and that the surrounding matrix material can be regarded as microstructurally stable.

4.1. Kinematics

In this subsection, we briefly review the necessary notations to describe the kinematics, summarize appropriate strain and stress tensors along with their associated invariants. Constitutive laws for isotropic and transversely isotropic hyperelastic materials will be derived. For a more complete discussion of nonlinear mechanics of continua we refer to the book by Holzapfel (Holzapfel, 2000).

To describe a deformation, we denote the stress-free reference configuration of the material by $\mathcal{B}_r$ and identify a generic material point by its position vector $\mathbf{X}$ relative to an arbitrary chosen origin. Application of mechanical forces deforms the body, so that the point $\mathbf{X}$ occupies the new position $\mathbf{x} = \boldsymbol{\chi}(\mathbf{X})$ in the deformed configuration $\mathcal{B}$. The vector field $\boldsymbol{\chi}$ describes the deformation of the body and assigns to each point $\mathbf{X}$ a unique position $\mathbf{x}$ in $\mathcal{B}$ and viceversa attributes a unique reference position $\mathbf{X}$ in $\mathcal{B}_r$ to each point $\mathbf{x}$. The deformation gradient tensor $\mathbf{F}$ relative to $\mathcal{B}_r$, is defined by

$$\mathbf{F} = \text{Grad} \boldsymbol{\chi}, \quad (1)$$

Grad being the gradient operator with respect to $\mathbf{X}$. The Cartesian components of $\mathbf{F}$ are $F_{i\alpha} = \partial x_i / \partial X_\alpha$, where x_i and X_α are the components of $\mathbf{x}$ and $\mathbf{X}$ respectively, and $i, \alpha \in \{1, 2, 3\}$. Roman indices are associated with $\mathcal{B}$ and Greek indices with $\mathcal{B}_r$. We also adopt the standard notation $J = \det \mathbf{F}$, with the convention $J > 0$.

The deformation gradient can be decomposed according to the unique polar decomposition

$$\mathbf{F} = \mathbf{R}\mathbf{U} = \mathbf{V}\mathbf{R}, \quad (2)$$

where $\mathbf{R}$ is a proper orthogonal tensor and $\mathbf{U}$ and $\mathbf{V}$ are positive definite and symmetric, respectively the right and left stretch tensors. These can be expressed in spectral form. For $\mathbf{U}$, for example, we have the spectral decomposition

$$\mathbf{U} = \sum_{i=1}^3 \lambda_i \mathbf{u}^{(i)} \otimes \mathbf{u}^{(i)}, \quad (3)$$

where the principal stretches $\lambda_i > 0$, $i \in \{1, 2, 3\}$, are the eigenvalues of $\mathbf{U}$, $\mathbf{u}^{(i)}$ are the (unit) eigenvectors of $\mathbf{U}$, and $\otimes$ denotes the tensor product. For volume preserving (isochoric) deformation, using equations (2) and (3), we have

$$J = \det \mathbf{F} = \det \mathbf{U} = \det \mathbf{V} = \lambda_1 \lambda_2 \lambda_3 \equiv 1. \quad (4)$$

Using the polar decomposition (2), we define

$$\mathbf{C}=\mathbf{F}^T\mathbf{F}=\mathbf{U}^2, \quad \mathbf{B}=\mathbf{F}\mathbf{F}^T=\mathbf{V}^2, \quad (5)$$

which denote the right and left Cauchy-Green deformation tensors respectively. According to the theory of invariants (Spencer, 1971), there exist three principal invariants for $\mathbf{C}$, equivalently $\mathbf{B}$, defined by

$$I_1=\text{tr } \mathbf{C}, \quad I_2=\frac{1}{2}[(\text{tr } \mathbf{C})^2 - \text{tr}(\mathbf{C}^2)], \quad I_3=\det \mathbf{C}=J^2, \quad (6)$$

where tr is the trace of a second-order tensor. Alternatively, in terms of principal stretches, the invariants I_1, I_2, I_3 are expressed as

$$I_1=\lambda_1^2+\lambda_2^2+\lambda_3^2, \quad I_2=\lambda_1^2\lambda_2^2+\lambda_1^2\lambda_3^2+\lambda_2^2\lambda_3^2, \quad I_3=\lambda_1^2\lambda_2^2\lambda_3^2. \quad (7)$$

In this study we consider silk fibers as a microstructural assembly of an oriented crystalline network embedded in an amorphous matrix. Let the unit vector $\mathbf{M}$ define the preferred direction of the crystalline network in the reference configuration $\mathcal{B}_r$. Additional invariants, denoted I_4 and I_5 and associated with the direction $\mathbf{M}$, are defined as

$$I_4=\mathbf{M} \cdot \mathbf{C}\mathbf{M}=\mathbf{F}\mathbf{M} \cdot \mathbf{F}\mathbf{M}=\mathbf{m} \cdot \mathbf{m}, \quad (8)$$

$$I_5=\mathbf{M} \cdot \mathbf{C}^2\mathbf{M}=\mathbf{m} \cdot \mathbf{B}\mathbf{m}, \quad (9)$$

where we introduced the notation $\mathbf{m} = \mathbf{F}\mathbf{M}$ to define the mapping of $\mathbf{M}$ under the deformation $\mathbf{F}$. There is a simple geometric interpretation for the invariant I_4 . The square root of I_4 provides the stretch of the material in the direction $\mathbf{M}$. No similar interpretation can be given for I_5 .

4.2. Hyperelastic Materials

The theory of hyperelasticity characterizes the elastic response of a body by a strain energy function W defined per unit volume in the reference configuration $\mathcal{B}_r$. For a homogeneous material, W depends only on the deformation gradient $\mathbf{F}$ and we write $W = W(\mathbf{F})$. In this paper we restrict attention to incompressible materials, subject to the constraint (4). Therefore, the nominal stress tensor $\mathbf{S}$ and the symmetric Cauchy stress tensor $\boldsymbol{\sigma}$ are given, respectively, by

$$\mathbf{S}=\frac{\partial W}{\partial \mathbf{F}}-p\mathbf{F}^{-1}, \quad \boldsymbol{\sigma}=\mathbf{F}\frac{\partial W}{\partial \mathbf{F}}-p\mathbf{I}, \quad (10)$$

where p is an arbitrary hydrostatic pressure. Equation (10) shows that for an incompressible material, the Cauchy stress $\boldsymbol{\sigma}$ and the nominal stress $\mathbf{S}$ are related by $\boldsymbol{\sigma} = \mathbf{F}\mathbf{S}$.

The unit vector $\mathbf{M}$ describes the preferred direction of a transversely isotropic material in the reference configuration $\mathcal{B}_r$. The material response is indifferent to an arbitrary rotation about the direction $\mathbf{M}$. Also the response is not altered by a change of direction from $\mathbf{M}$ to $-\mathbf{M}$.

Following the analysis of such materials given by Spencer (Spencer, 1971) and Ogden (Ogden, 1972), we define a transversely isotropic material as one for which the strain energy W is an isotropic function of the two tensors $\mathbf{F}$ and $\mathbf{M} \otimes \mathbf{M}$. For an incompressible material $I_3 \equiv 1$ and the form of W is reduced to dependence on the four independent invariants I_1, I_2, I_4, I_5 . We write $W = W(I_1, I_2, I_4, I_5)$.

In order to obtain the explicit expressions of the nominal stress tensor $\mathbf{S}$ and the associated Cauchy stress tensor $\boldsymbol{\sigma}$ the derivatives of the strain invariants with respect to $\mathbf{F}$ are needed. Following standard derivation rules, these are given by

$$\frac{\partial I_1}{\partial \mathbf{F}} = 2\mathbf{F}^T, \quad \frac{\partial I_2}{\partial \mathbf{F}} = 2(I_1\mathbf{F}^T - \mathbf{F}^T\mathbf{F}\mathbf{F}^T), \quad (11)$$

$$\frac{\partial I_4}{\partial \mathbf{F}} = 2\mathbf{M} \otimes \mathbf{F}\mathbf{M}, \quad \frac{\partial I_5}{\partial \mathbf{F}} = 2(\mathbf{M} \otimes \mathbf{F}\mathbf{C}\mathbf{M} + \mathbf{C}\mathbf{M} \otimes \mathbf{F}\mathbf{M}). \quad (12)$$

A direct calculation of (10), using equations (11) and (12) leads to

$$\mathbf{S} = 2(W_1 + I_1 W_2)\mathbf{F}^T - 2W_2\mathbf{C}\mathbf{F}^T + 2W_4\mathbf{M} \otimes \mathbf{F}\mathbf{M} + 2W_5(\mathbf{M} \otimes \mathbf{F}\mathbf{C}\mathbf{M} + \mathbf{C}\mathbf{M} \otimes \mathbf{F}\mathbf{M}) - p\mathbf{F}^{-1}, \quad (13)$$

and

$$\boldsymbol{\sigma} = 2(W_1 + I_1 W_2)\mathbf{B} - 2W_2\mathbf{B}^2 + 2W_4\mathbf{m} \otimes \mathbf{m} + 2W_5(\mathbf{m} \otimes \mathbf{B}\mathbf{m} + \mathbf{B}\mathbf{m} \otimes \mathbf{m}) - p\mathbf{I}, \quad (14)$$

where the abbreviations $W_i = \partial W / \partial I_i$, $i = 1, 2, 4, 5$ have been introduced. When the dependence on I_4 and I_5 in equations (13) and (14) is omitted, the associated expressions for an isotropic material are obtained.

Traditionally, for *transversely isotropic materials* the strain energy is given by the sum of two contributions, one associated with the isotropic properties of the base matrix and the second with the anisotropy being generated by the embedded fibers. Therefore we can write

$$W = W_{\text{iso}}(I_1, I_2) + W_{\text{fib}}(I_4, I_5), \quad (15)$$

where the term W_{iso} represents an isotropic matrix material and W_{fib} accounts for the oriented fibrous network. Following the simplification in (Holzapfel, 2000), we reduce the number of invariants and consider

$$W = W_{\text{iso}}(I_1) + W_{\text{fib}}(I_4), \quad (16)$$

which still provides sufficient flexibility to capture the anisotropic response. The expressions of the nominal stress (13) and Cauchy stress (14) become

$$\mathbf{S} = 2W_1 \mathbf{F}^T + 2W_4 \mathbf{M} \otimes \mathbf{F} \mathbf{M} - p \mathbf{F}^{-1}, \quad (17)$$

$$\boldsymbol{\sigma} = 2W_1 \mathbf{B} + 2W_4 \mathbf{m} \otimes \mathbf{m} - p \mathbf{I}. \quad (18)$$

4.3. Modeling of virgin material

Biological soft tissues, in general, tend to stiffen rapidly as fibers are recruited and aligned along the loading directions. In view of the microstructural representation of silk fibers described in (Shen et al., 1998; Krasnov et al., 2008), we propose to use the neo-Hookean model to represent the amorphous matrix and the exponential formulation proposed by Horgan and Saccomandi (Horgan et al., 2004) for the crystalline network. Following (16), the two-term energy function for the virgin material is given by

$$W = \frac{\mu_{\text{iso}}}{2} (I_1 - 3) - \frac{\mu_{\text{fib}}}{n} J_m \ln \left[1 - \frac{(I_4 - 1)^n}{J_m} \right], \quad (19)$$

where μ_{iso} is the shear modulus of the amorphous matrix in the reference configuration, μ_{fib} measures the increase in stiffness due to the oriented crystalline network, n is a material parameter and J_m is a dimensionless coefficient to represent the degree of rigidity. Note that for $J_m \rightarrow \infty$, the second term of the strain energy formulation reduces to

$$W_{\text{fib}} = \frac{\mu_{\text{fib}}}{n} (I_4 - 1)^n, \quad (20)$$

which for $n = 2$ is often called the standard reinforcing model (Qiu and Pence, 1997; Merodio and Ogden, 2005; Horgan et al., 2004).

4.3.1. Loading—Using the energy formulation (19), the explicit expression for the nominal stress (17) of the virgin material becomes

$$\mathbf{S} = \mu_{\text{iso}} \mathbf{F}^T + 2\mu_{\text{fib}} \frac{J_m (I_4 - 1)^{n-1}}{J_m - (I_4 - 1)^n} \mathbf{M} \otimes \mathbf{F} \mathbf{M} - p \mathbf{F}^{-1}. \quad (21)$$

Similarly, from equation (18) we get the Cauchy stress tensor

$$\boldsymbol{\sigma} = \mu_{\text{iso}} \mathbf{B} + 2\mu_{\text{fib}} \frac{J_m (I_4 - 1)^{n-1}}{J_m - (I_4 - 1)^n} \mathbf{m} \otimes \mathbf{m} - p \mathbf{I}, \quad (22)$$

where $\mathbf{m}$ is the preferred direction in the current configuration, see equation (8).

4.3.2. Uniaxial loading in the preferred direction—In the simple tension specialization the preferred direction coincides with the loading direction. We take the principal stretches $\lambda_2 = \lambda_3$ and use the notation

$$\lambda_1 = \lambda, \quad \lambda_2 = \lambda^{-1/2}, \quad (23)$$

satisfying the incompressibility condition (4). From equations (7) and (8), we have

$$I_1 = \lambda^2 + 2\lambda^{-1}, \quad I_4 = \lambda^2, \quad (24)$$

which shows that the strain energy function (for virgin materials) depends only on the independent stretch λ . Introducing the notation $\widehat{W}(\lambda)$ gives the correspondence

$$\widehat{W}(\lambda) = W(I_1, I_4). \quad (25)$$

Writing the strain energy (19) as a function of λ , we obtain

$$\widehat{W}(\lambda) = \frac{\mu_{\text{iso}}}{2}(\lambda^2 + 2\lambda^{-1} - 3) - \frac{\mu_{\text{fib}}}{n} J_m \ln \left[1 - \frac{(\lambda^2 - 1)^n}{J_m} \right]. \quad (26)$$

The single non-zero stress component is in the fiber direction and $\sigma_2 = \sigma_3 = 0$. Using (22), the Cauchy stress associated with λ has the form

$$\sigma = \lambda \frac{d\widehat{W}(\lambda)}{d\lambda} = \mu_{\text{iso}}(\lambda^2 - \lambda^{-1}) + 2\mu_{\text{fib}} \lambda^2 \frac{J_m(\lambda^2 - 1)^{n-1}}{J_m - (\lambda^2 - 1)^n}, \quad (27)$$

where the material parameters μ_{iso} , μ_{fib} , J_m and n are determined using the experimental load-deformation data of the virgin fiber material.

4.4. Modeling degradation of polymer networks

In this section we modify the transient network theory, which was originally proposed by Rajagopal and Wineman (Rajagopal and Wineman, 1992) to model deformation induced microstructural changes in isotropic elastic materials. Each increment in deformation induces a breakage of bonds, which instantly reassemble to form a new degraded network. The transient network theory to recently generalized to treat transversely isotropic materials with microstructural changes restricted to the fibrous network and with the surrounding matrix material regarded as microstructurally stable (Demirkoparan et al., 2009). Interestingly, an equivalent idea was used to develop parallel elastoplastic models to represent the mechanical behavior of inelastic materials (Nelson and Dorfmann, 1995).

In the current study we take silk fibers to consist of an organized network of interconnected crystalline domains surrounded by amorphous regions as described in detail in (Shen et al., 1998; Krasnov et al., 2008). In addition, we consider that the breakage and reformation is restricted to the crystalline network and that the surrounding matrix material can be regarded as microstructurally stable. Degradation of the material is initiated at a given stretch λ_s and completed when $\lambda = \lambda_f$. These are material parameters whose values are determined using available data and satisfy the inequalities $1 < \lambda_s < \lambda_f$. Following (Demirkoparan et al., 2009),

we define functions $\beta(\lambda)$ and $\alpha(\lambda)$ that describe respectively the degradation of the primary network and the subsequent reassembly process. We assume that both functions depend on the current stretch λ with $\lambda_s < \lambda < \lambda_f$. In particular, $\beta(\lambda)$ gives the fraction of the original network that is still active when the fiber is stretched by an amount λ , correspondingly the function $\alpha(\lambda)$ gives the amount of secondary network that has been assembled. For $\lambda < \lambda_s$ no degradation in the original microstructure occurs, for $\lambda = \lambda_f$ degradation is completed and for $\lambda > \lambda_f$ only the secondary network remains active.

The secondary network is assembled in the deformed configuration and is stress-free at the time of formation. It is convenient to denote the current length of a segment of the secondary network by l , the length when it is formed by $\hat{l}$ and the reference length in the virgin state by l_r . Then, the total stretch with respect to the original reference configuration is $\lambda = \hat{\lambda}\check{\lambda}$, where $\check{\lambda} = \hat{l}/l_r$ is the stretch at reassembly that remains constant for future elongations and $\hat{\lambda} = l/\hat{l}$ is the stretch of the network evaluated with respect to the new reference length $\hat{l}$. At the instant of its formation we have $\hat{\lambda} = 1$ and therefore no residual stress exist in the newly formed network. We further introduce the notation λ_{nat} to define the new natural configuration after unloading. It is the stretch from the original reference position that defines the new stress-free configuration. In other words, after tensile loading for example, the unloaded configuration is changed from $\lambda = 1$ to $\lambda_{\text{nat}} > 1$. Also, it is reasonable to assume that the preferred direction of the reassembled network coincides with the direction of the crystalline network in the original assembly, i.e. it remains along the fiber axis. For a detailed discussion of the transient network theory in the 3-D context we refer to (Demirkoparan et al., 2009).

For an extension λ with $\lambda_s < \lambda < \lambda_f$ the network degrades and reassembles as specified by the functions β and α , respectively. The first term on the right hand side of the energy formulation (26) represents the amorphous regions, which are assumed to be microstructurally stable. The remaining part of the energy, accounting of the oriented crystalline network, needs to be modified to include degradation. We write

$$W_{\text{fib}} = \frac{\mu_{\text{fib}}}{n} J_m \left[-\beta \ln \left(1 - \frac{(\lambda^2 - \lambda_{\text{nat}}^2)^n}{J_m} \right) - \int_{\lambda_s}^{\lambda} \alpha(\check{\lambda}) \ln \left(1 - \frac{(\hat{\lambda}^2 - \lambda_{\text{nat}}^2)^n}{J_m} \right) d\check{\lambda} \right], \quad (28)$$

where we recall that $\hat{\lambda} = \lambda\check{\lambda}$ and where the notation $\alpha(\check{\lambda})$ indicates the dependence of α on $\check{\lambda}$. Equation (28) also shows that the mechanical property μ_{fib} of the primary and reassembled networks coincide, an assumption we believe does not change the main findings of this study. During the degradation process when $\lambda_s < \lambda < \lambda_f$, using expression (28), the nonzero Cauchy stress component has the form

$$\sigma = \mu_{\text{iso}}(\lambda^2 - \lambda^{-1}) + 2\mu_{\text{fib}} \left[\beta \lambda^2 \frac{J_m(\lambda^2 - \lambda_{\text{nat}}^2)^{n-1}}{J_m - (\lambda^2 - \lambda_{\text{nat}}^2)^n} + \int_{\lambda_s}^{\lambda} \alpha(\check{\lambda}) \hat{\lambda}^2 \frac{J_m(\hat{\lambda}^2 - \lambda_{\text{nat}}^2)^{n-1}}{J_m - (\hat{\lambda}^2 - \lambda_{\text{nat}}^2)^n} d\check{\lambda} \right]. \quad (29)$$

Degradation and reassembly occurs in the deformed configuration and therefore newly formed networks have a different reference length. This is evidenced by the use of $\hat{\lambda}$ in the assembly process. In other words, the stretch of the secondary network is determined using $\hat{l}$ as the reference length, at reassembly we therefore have $\hat{\lambda} = 1$ and the contribution to the stress vanishes.

Degradation of the crystalline network takes place for the stretch interval $\lambda_s < \lambda < \lambda_f$ where the function β is monotonically decreasing from one to zero. The strain energy formulation after degradation is completed has the form

$$W = \frac{\mu_{iso}}{2}(\lambda^2 + 2\lambda^{-1} - 3) - \frac{\mu_{fib}}{n} J_m \int_{\lambda_s}^{\lambda_f} \alpha(\check{\lambda}) \ln \left(1 - \frac{(\check{\lambda}^2 - \lambda_{nat}^2)^n}{J_m} \right) d\check{\lambda}, \quad (30)$$

and the corresponding stress is given by

$$\sigma = \mu_{iso}(\lambda^2 - \lambda^{-1}) + 2\mu_{fib} \int_{\lambda_s}^{\lambda_f} \alpha(\check{\lambda}) \check{\lambda}^2 \frac{J_m (\check{\lambda}^2 - \lambda_{nat}^2)^{n-1}}{J_m - (\check{\lambda}^2 - \lambda_{nat}^2)^n} d\check{\lambda}. \quad (31)$$

Finally, we note that during unloading no further degradation occurs and the material is best characterized as nonlinear elastic with material properties determined by the maximum extension seen during the prior loading sequence.

4.4.1. Kinetics of breakage and reformation—To describe the kinetic of degradation and reassembly we use, for convenience, the formulations derived in (Demirkoparan et al., 2009). In particular, we assume that the reassembly process accounts for all degraded material and therefore have the conservation requirement

$$\beta(\lambda) + \int_{\lambda_s}^{\lambda} \alpha(\check{\lambda}) d\check{\lambda} = 1. \quad (32)$$

It can be shown that a possible choice for β is

$$\beta(\lambda) = \begin{cases} 1 & \text{for } \lambda < \lambda_s, \\ \frac{2(\lambda - \lambda_f)^2(\lambda - \lambda_p)}{(\lambda_f - \lambda_s)^3} & \text{for } \lambda_s \leq \lambda \leq \lambda_f, \\ 0 & \text{for } \lambda > \lambda_f, \end{cases} \quad (33)$$

where $\lambda_p = (3\lambda_s - \lambda_f)/2$. The reassembly is then given by

$$\alpha(\lambda) = \begin{cases} 1 & \text{for } \lambda < \lambda_s, \\ \frac{6(\lambda_f - \lambda)(\lambda - \lambda_s)}{(\lambda_f - \lambda_s)^3} & \text{for } \lambda_s \leq \lambda \leq \lambda_f, \\ 0 & \text{for } \lambda > \lambda_f. \end{cases} \quad (34)$$

5. Numerical results

To validate constitutive models (27), (29) and (31), together with kinetic equations (33) and (34), we find the magnitude of the seven adjustable parameters μ_{iso} , μ_{fib} , n , J_m , λ_s , λ_f and λ_n by matching experimental data of material degradation due to Protease XIV and cyclic loading, see Figure 3. The observations for α -chymotrypsin are not used, but equivalent steps could be followed to find the values of the corresponding material model parameters.

The transient network theory could easily be extended to include the amorphous domains as well, but a number of additional model parameters would need to be introduced. For simplicity, we therefore consider the amorphous network to be mechanically stable with stiffness $\mu_{\text{iso}} = 100$ MPa for the duration of the test. We also assume that the degradation of the crystalline network starts with stretching of the fiber, i.e. $\lambda_s = 1$, and progresses with each increment in deformation. Therefore, the five remaining adjustable parameters to match the virgin material response and degradation over time are μ_{fib} , n , J_m , λ_f and λ_n .

We begin with matching the loading and unloading data denoted Day 0 and shown in Figure 3, and determine the stiffness of the crystalline network μ_{fib} , the exponent n and the degree of rigidity J_m . Equation (27) shows that these parameters, in addition to μ_{iso} , completely define the behavior of the virgin material. First, we match the slope of the stress-deformation response at small strains and find $\mu_{\text{fib}} = 49000$ MPa. Afterwards, we approximate the exponential stiffening during first loading and find $n = 2.73$ and $J_m = 0.005$ for a best fit. The numerical results obtained, using equation (27) with the known values of model parameters, show that some degradation occurs at day 0 during larger strains. To match the experimental data we gradually increase, starting from 1, the value λ_f and find the best match for $\lambda_f = 1.063$. We recall that this parameter defines the maximum stretch where the degradation of the crystalline network is completed. For an elongation $\lambda < \lambda_f$ degradation is not completed and a fraction of the original network remains active. The experimental data in Figure 2 also show that on day 0, upon unloading, no permanent set has yet occurred and therefore $\lambda_{\text{nat}} = 1$.

We have now determined the values of all material model parameters to describe the loading and unloading response on day 0. To approximate the degradation of silk fibers subjected to Protease XIV and a daily cyclic loading-unloading sequence, we now fix the values of n and J_m and use the available data to determine the three remaining parameters μ_{fib} , λ_f and λ_n .

Using a similar process as described above, we determine the values of the remaining parameters by approximating the stress-strain data recorded on days 9 and 12. We first determine the value of μ_{fib} to match the slope of the stress-stretch behavior at small strains. Afterwards we gradually increase the value of λ_f starting from 1.063, which was found previously for data recorded on day 0. The objective is to match the increase in stiffening of the fibers with increasing deformation. Finally, the permanent set in the fiber, once the applied load is removed, is approximated by adjusting the value of λ_n . The numerical values of the model parameters approximating the data obtained for days 0, 9 and 12 are summarized in Table 1 and shown in Figures 5–7. It is now easy to determine the model parameters for the remaining data corresponding to days 5, 7 and 14. In particular, Figure 5 shows that the mechanical stiffness of the degrading crystalline network decreases linearly with time. Figure 6 shows the variation of λ_n during the duration of the test. It shows that the permanent set increases monotonically with time of exposure. Finally, Figure 7 indicates an increase over time in the maximum extension necessary to fully degrade the crystalline network and the existence of a critical time at which a rapid increase in the material degradation occurs.

The numerical response of six loading-unloading cycles, using the constitutive formulation described in the previous section, in combination with the numerical values of the model parameters, is depicted in Figure 8. Fair agreement between the numerical results and experimental data is achieved, see Figure 3. These results justify the use of the transient network model to describe the degradation process of silk fibers in the presence of Protease XIV.

6. Concluding Remarks

In this study we provide new experimental data to characterize the degradation process of silk fibers due to combined enzymatic treatment and cyclic loading-unloading. In particular, we exposed fibers to Phosphate Buffered Saline (PBS), to Protease XIV and to α -chymotrypsin.

The data show that exposure to enzymes reduces the ultimate strength of the material, but leaves the stiffness unchanged. Experimental results further show that the addition of cyclic loading-unloading during incubation induces significant amounts of stress softening and permanent set, both for Protease XIV and for α -chymotrypsin. The presence of PBS did not alter the mechanical response.

A constitutive equation has been developed for the degradation behavior in the presence of enzymes and at finite strain. The silk fibers are treated as an organized fibrous network of interconnected crystalline domains surrounded by amorphous regions (Shen et al., 1998; Krasnov et al., 2008). With reference to the concept of transient networks (Rajagopal and Wineman, 1992), each increment in deformation induces a breakage of bonds, which instantly reassemble to form a new degraded network. The secondary network is assembled in the deformed configuration and is stress-free at the time of formation. Upon removal of the applied load, the network does not return to the original reference configuration, but to a new configuration accounting of residual deformations. In the application of the theory, we assumed that the microstructural changes are restricted to the fibrous network and that the surrounding matrix material is microstructurally stable. Fair agreement between the numerical results and experimental data is achieved.

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Figure 1.

Results from monotonic extension tests of silk fibers incubated with 1 mg/mL Protease XIV at 37 °C. The results show the average of $n=4$ fibers independently assessed and the final data point of each curve as the maximum stress recorded. “Day 0” corresponds to samples immersed in PBS prior to testing without incubation in Protease XIV. Data denoted “PBS” correspond to samples immersed in PBS for 14 days.

Figure 2.

Experimental data showing the loading-unloading response of a silk fiber during 14 days exposure to Phosphate Buffered Saline (PBS). Data shown correspond to the 30th loading-unloading cycle of a daily 60 cycle loading regime. Overlapping graphs are *omitted* for clarity.

Figure 3.

Experimental data showing significant degradation of a silk fiber exposed to Protease XIV and subject to a daily sequence of 60 loading-unloading cycles. The 30th cycle on days 0 (top curve), 5, 7, 9, 12, and 14 (bottom curve) is shown.

Figure 4.

Experimental data showing the degradation of a silk fiber during a 14 day exposure to α -chymotrypsin and to 60 daily loading-unloading cycles. Results shown are for the 30th cycle on days 0 (top curve), 5, 7, 9, 12, and 14 (bottom curve). Overlapping graphs are *omitted* for clarity.

Figure 5. Variation of mechanical stiffness over time of a crystalline network in silk fibers that are exposed to Protease XIV and subject to 60 loading-unloading cycles per day. Maximum stretch during cyclic loading is $\lambda = 1.025$.

Figure 6.

The monotonic increase of λ_n over time indicating an increase in permanent set of silk fibers with time of exposure to Protease XIV and periodic cyclic loading.

Figure 7.

Variation of the material parameter λ_f over time indicating the maximum extension necessary to fully degrade the crystalline network in silk fibers that are exposed to Protease XIV and subject to 60 loading-unloading cycles per day. The maximum stretch during cycling is $\lambda = 1.025$.

Figure 8.

Numerical results showing the loading-unloading response of a degrading silk fiber on days 0 (top curve), 5, 7, 9, 12 and 14 (bottom curve). The results show fair agreement with experimental observation of silk fibers exposed to Protease XIV and subject to 60 loading-unloading cycles per day, see Figure 3.

Values of material model parameters to describe degradation of silk fibers over time when subject to cyclic loading and exposed to Protease XIV. The parameters μ_{iso} and μ_{fib} are given in MPa.

Table 1

Days	μ_{iso}	μ_{fib}	n	J_m	λ_s	λ_f	λ_n
0	100	49000	2.73	0.005	1.0	1.063	1.0
5	100	39000	2.73	0.005	1.0	1.075	1.0015
7	100	35000	2.73	0.005	1.0	1.080	1.0025
9	100	31000	2.73	0.005	1.0	1.100	1.0040
12	100	26000	2.73	0.005	1.0	1.150	1.0064
14	100	22500	2.73	0.005	1.0	1.150	1.0085