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Equal and local-load-sharing micromechanical models for collagens: Quantitative comparisons in response of non-diabetic and diabetic rat tissue

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Abstract

Chemical crosslinks in collagens resulting from binding of advanced glycation end-products, have long been presumed to alter the stiffness and permeability of glycated tissues. Recently, we developed a stochastic mechanical model for the response and failure of uniaxially deformed sciatic nerve tissue from diabetic and control rats. Here, we use our model to determine the likely correlation of fibril glycation with failure response, by quantifying statistical differences in their response. Our four-parameter model describes both the non-linear toe region and non-linear failure region of these tissues; the four parameters consist of (1) collagen fibril alignment, (2) fiber bundle waviness, (3) Weibull shape parameter for fibrillar strength, and (4) modulus-normalized Weibull scale parameter for fibrillar strength. Using an equal load sharing model we find that diabetic and control tissues had shape parameters of 9.88 ± 5.50 and 4.33 ± 3.67 ($p = 0.043$), respectively, and scale parameters of 0.28 ± 0.07 and 0.58 ± 0.25 ($p = 0.033$), respectively, implying that the diabetic tissue behaves in a more brittle manner, consistent with more highly crosslinked fibrils. We conclude that biochemical crosslinking directly affects measured mechanical properties. Further, this mechanical characterization may prove useful in mapping alterations in stiffness and permeability observed in glycated tissues.

Keywords: Nerve; Mechanics; Collagen; Diabetes; ECM

1. Introduction

A clear pathway from the binding of advanced glycation end-products (AGEs) to alterations in the mechanical response of collagenous tissue has not yet been directly established, though they are clearly linked. Glycation-induced crosslinkings of fibrillar collagens of types I, III, V and XI [2–7] have been implicated, for example, in stiffening of skin [8,9] and reduced fracture toughness in bone [10]. Greater duration and intensity of exposures to glucose have been shown to amplify the effects of collagen glycation [11–13].

Nerve tissue contains ~10–16% collimated collagen by mass fraction [14–16]. This tissue undergoes glycation [17,18], and exhibits altered mechanical properties, which can be modeled using micromechanics [18,19]. The gross mechanical properties of peripheral nerve [20,21] and collagen fibrils [22,23] have been quantified, but until our own work, there was apparently no model correlating glycation with mechanical properties at the tissue scale. Our methodology [18,19] was an application of classical bundle theory, applied to the fibrillar collagen of the nerve sheath [24–26]. The model was developed for the fibril scale (Fig. 1), at which the individual fibrils may be modeled as spatially periodic and finite in length. The concept underlying this

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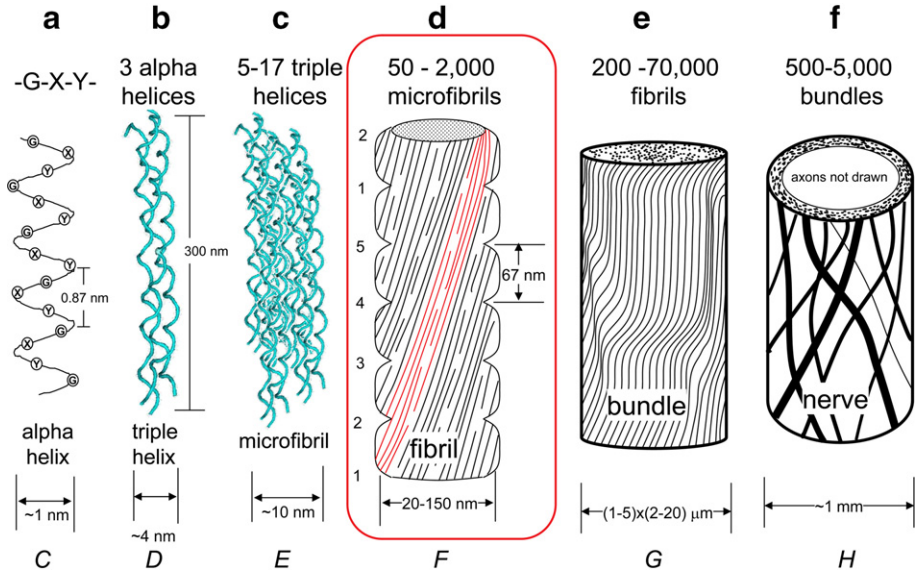

Fig. 1. A series of figures from molecular scale to tissue scale. The letters *C–H* correspond to failure functions [24,25] used to predict failure at a larger scale based on known properties at a smaller scale. The function *F* was chosen to represent the fibril scale and is the scale of interest for the present work. In (a) a left-handed triple helix of a fibrillar collagen is depicted with glycine occupying every third position, see e.g., Refs. [81,82]. In (b) three triple helices, see e.g., Ref. [83], self-assemble into (c) microfibrils of varying size prior aggregating into (d) fibrils [55,84]. These fibrils are then organized into (e) wavy tape-like bundles in the epineurium that encase (f) whole nerve [50,85]. Dimensions given for fibrils, bundles and whole nerve are those of rat sciatic nerve.

present work is based on weakest-link scaling: a structure comprised of distinct subcomponents placed in series fails when one subcomponent fails. For structures comprised of parallel subcomponents, all subcomponents in a given link must fail before the entire structure fails. Here, we model nerve tissue as a set of load-supporting collagen fibrils in parallel.

More generally, the order in which neighbors of broken fibrils rupture depends upon the nature of the "matrix;" in this case, the matrix is the ground substance, which can be assumed to have negligible mechanical effect in collimated, collagen-reinforced nerve tissues. For glycated tissues, however, collagens can be assumed to exhibit substantial crosslinking under these conditions, and so, the effect is similar to that of a matrix of low contrast ratio to the reinforcing phase (i.e., similar mechanical modulus). One extreme strategy for modeling sequential rupture in such a material is to assume that all surviving fibrils carry load equally (equal load sharing, or ELS). The other extreme is to assume that only the fibrils in the immediate vicinity of the broken fibril assume all overload (an example of local load sharing, or LLS). These models correspond, respectively, to a matrix of low or high contrast ratio. With glycation, it can be reasonably hypothesized that response of collimated collagens would be closer to LLS, due to increased correlation of fibrils.

In this work, we present a parametric constitutive failure model for peripheral nerve, to test this hypothesis, using the following key variables: fibril waviness, as defined by the ratio between the spatial amplitude and fibril periodicity, fibril angle with respect to the primary tissue axis, Weibull shape parameter, and Weibull scale parameter. Other variables included are collagen fibril modulus, tissue scale collagen density, and microscale packing density [19]. The model is capable of capturing the full behavior of the non-linear behavior of the nerve tissue from the toe region, through the linear region and up to failure. The fibril waviness and fibril angle capture the shape of the toe region, while the Weibull scale and shape factors describe the failure region.

1.1. AGE binding of collagens

The basis for the presumably increased mechanical correlation of glycated fibrils is the known increase in AGE binding in blockage of binding sites of extracellular matrix (ECM) proteins such as collagen IV and laminin, impeding cell adhesion, growth, and tissue remodeling [27]. AGE-specific fluorescence and enzyme-linked immunosorbent assay (ELISA)-based binding have been shown to increase with time in the coronary collagen of diabetes-induced rats [28]. ELISA and solubility assays have been used to show that collagen crosslinks form both in the presence and absence of either free glucose or oxygen or a combination of glucose and oxygen [4]. Overall, extensive chromatography experiments with high performance liquid chromatography have resulted in the identification of over 30 distinct AGEs [29,30]. Indeed, the presence of AGEs coincident with glucose exposure has been shown to stiffen human skin exposed to glucose-6-phosphate *in vitro* by as much as 80% [9] and rat tail tendon by 100% [31] *in vitro*, and to diminish stiffness by 50% and strength by 29% in healing rat femoral bone, *in vivo* [10]; the crosslinks that are presumably responsible for stiffening skin are also responsible

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