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Multi-scale modeling of soft fibrous tissues based on proteoglycan mechanics

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ABSTRACT

Collagen in the form of fibers or fibrils is an essential source of strength and structural integrity in most organs of the human body. Recently, with the help of complex experimental setups, a paradigm change concerning the mechanical contribution of proteoglycans (PGs) took place. Accordingly, PG connections protect the surrounding collagen fibrils from over-stretching rather than transmitting load between them. In this paper, we describe the reported PG mechanics and incorporate it into a multi-scale model of soft fibrous tissues. To this end, a nano-to-micro model of a single collagen fiber is developed by taking the entropic-energetic transition on the collagen molecule level into account. The microscopic damage occurring inside the collagen fiber is elucidated by sliding of PGs as well as by over-stretched collagen molecules. Predictions of this two-constituent-damage model are compared to experimental data available in the literature.

1. Introduction

Collagen and proteoglycans are the main contributors to mechanical properties of tendon and other soft collagenous tissues. They ensure structural integrity and strength of many organs of the human body and have been subject of intensive research since decades. In particular, PG interactions with each other are still an open issue. While proteoglycan chains their self are very weak from the mechanical point of view, the collagen fibrils demonstrate a very stiff material behavior. However, since collagen fibrils are embedded in a proteoglycan rich matrix, the interaction between these two components is of special interest in regard to the macroscopic response. By utilizing modern microscopic techniques, new insights towards understanding of proteoglycans mechanics have recently been delivered (Rigozzi et al., 2013).

So far, two mutually contradicting theories of fibril-PGs mechanics have been proposed. The first theory describes the PGs as mechanical cross-linkers between adjacent collagen fibrils (Scott, 1991, 2003; Cribb and Scott, 1995; Sasaki and Odajima, 1996; Robinson et al., 2005) and accordingly attributes them the capability of transmitting loads between fibrils. In contrast, Fessel and Snedeker (2009, 2011) consider the PGs and their associated glycosaminoglycan (GAG) sidechains (see Figs. 3 and 4) as an interjacent medium with zero stiffness under tension. They conducted mechanical tests with a native and GAG depleted tendon (Fessel and Snedeker, 2009) and compared the results against a 3D FE model based on a mediated inter fibril-load shearing assumption (Fessel and Snedeker, 2011). The experimental results suggest that the digestion of GAG sidechains does not weaken the tendon under tension. Instead, the stiffness even slightly increases at the strain level of approximately 4% and above. In contrast, the FE analysis predicts a weakening of the stress response by a reduction of GAGs, which supports the theory that PGs do not contribute to the load transmission between adjacent fibrils.

Further, interventions by using optical-mechanical measurements focusing on the mechanical properties of GAGs were recently reported by Rigozzi et al. (2009, 2013). Utilizing the atomic force microscopy (AFM) they observed a higher fibril strain in Achilles tendon with a reduced PG content in comparison to native Achilles tendon samples. Thus, they suggested that GAGs are responsible for the interfibrillar sliding only. By this means, PG chains act as intermediate bumpers between adjacent fibrils and protect them from rupturing. This observation supports the hypothesis, further referred to as *slide-stuck* theory, that the absence of an appropriate amount of GAGs contributes to the friction between fibrils and thus increases the fibril strain level. Hence, due to this protective coating surrounding the fibrils, the fibril stretch has to be smaller than the tissue/macro-stretch. Taking advantage of this structure, the tissues have an additional mechanism to reduce the risk of permanent damage. The stretch of longer fibrils can, however, increase for higher fibril lengths due to higher tangential contact forces. Recently, a similar proposition

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has also been made by Fessel and Snedeker (2011), who analyzed fibril lengths within the aforementioned FE-study.

Few studies have been published on the constitutive modeling of collagen, proteoglycans and their interaction with each other. In one of the first studies (Gasser, 2011) PGs and fibrils are smeared into a medium with a single strain energy while the PG-bridges are used as a motivation for a phenomenological damage function. By this means, a model based on the assumptions of Scott (2003) and utilizing mirco-mechanically motivated damage functions in a statistical framework (Schmidt et al., 2014) was proposed. Other works dealing with micro-mechanically motivated constitutive laws of soft fibrous tissues focus more on the precise description of collagen fibrils and on the collagen fiber curvature (see, e.g. Marino and Vairo, 2014). However, modeling of slipping at biological interface, in particular, between hydroxyapatite and tropocollagen for hard biological tissues was investigated in Fritsch et al. (2009) and further supported by molecular dynamics studies in Qu et al. (2015).

To the best of our knowledge, the *slide-stuck* theory of the proteoglycan–fibril interaction has not so far been addressed in the framework of constitutive modeling. Hence, in the current paper a constitutive formulation of this theory with the ability to capture the characteristics reported recently in Rigozzi et al. (2013) is proposed. One of these characteristics is in particular a stiffening of the macro-stress response under a decreasing fibril surrounding PG density. The paper is organized as follows: Section 2 presents a brief overview of the mechanics of a single collagen molecule. Section 3 enhances the tropocollagen (TC) molecule theory to the overlying structure of collagen fibrils. Section 4 is devoted to the PG–fibril interaction. A generalized constitutive model of soft fibrous tissues is proposed in Section 5. Finally, in Sections 6 and 7 the constitutive equations are derived and model predictions are compared against experimental data available in the literature.

2. Mechanics of a single TC molecule

Tropocollagen is a triple helical arrangement of three coiled collagen-protein chains. The chains are connected together by a certain amount of hydrogen bonds. By a D-banding pattern TC molecules are assembled to their overlying structure referred to as collagen fibrils. To provide their integrity, the TCs are attached to each other at the ends by covalent bonded cross-linkers. Collagen fibrils show up with a molecular gap in their periodical assembly, where the molecules have the highest density of disorder (Fratzl et al., 1998).

Under tension the molecules become aligned and start to straighten out. During this process, the molecular disorder reduces and the entropic force increases until the extended helix length is reached (Bozec and Horton, 2005; Buehler and Wong, 2007), see Fig. 1. The energy accumulated in the low force regime of a TC arises from the bending stiffness based on thermally induced entropic elasticity. It has been shown before (Bozec and Horton, 2005; Buehler, 2006) that this behavior can be well captured by applying the classical worm-like chain (WLC) formulation (Kratky, 1955), where the total energy of bending deformations is due to thermal fluctuations. Hence, we utilize a force–extension relation for the WLC given by the interpolation function (Marko and Siggia, 1995)

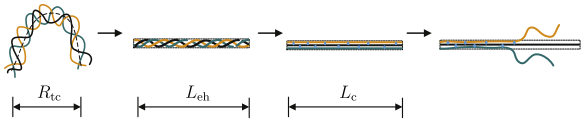

Fig. 1. Illustration of the different deformation states of a single TC molecule: from the bent structure in the reference configuration, over the straighten out state characterized by the extended helix length L_{eh} and the total uncoiled state with the contour length L_c to the damaged configuration.

$$F_{wlc}(r_{tc}, L_{eh}) = \frac{k_B T}{l_p}\left[\frac{1}{4}\left(1-\frac{r_{tc}}{L_{eh}}\right)^{-2} - \frac{1}{4} + \frac{r_{tc}}{L_{eh}}\right]. \tag{1}$$

Three material parameters included therein are the persistence length l_p a quantity displaying the bending stiffness, the extended helix length L_{eh} (see Fig. 1) and the current end-to-end length r_{tc} given as

$$r_{tc} = R_{tc}\,\lambda_{tc}, \tag{2}$$

where R_{tc} denotes the reference end-to-end length and λ_{tc} is the molecular stretch.

In higher force regimes, when an external applied force is sufficiently big to suppress the thermal fluctuations, the energy regime changes over to the intrinsic elasticity regime of a TC. The potential of the external force F_{hx} can be given by (Kamien and Lubensky, 1997; and Marko, 1997)

$$\Pi_{hx} = \frac{1}{2}\frac{C}{L_{eh}}\theta^2 + \frac{1}{2}\frac{S}{L_{eh}}R_{tc}^{*2} + \frac{g\,\theta}{L_{eh}}R_{tc}^{*} - F_{hx}R_{tc}^{*}, \tag{3}$$

where $R_{tc}^{*} = (R_{tc} - L_{eh})$ denotes the elongation after reaching the extended helix length L_{eh}. The first term is due to the twisting stiffness of a helix with the twist rigidity C, the second term captures the stretching of single protein chains with the stretch modulus S and the third term is a twist-stretch coupling expression with the factor g. These terms are necessary for a unique description of DNA within the linear theory of double stranded DNA (Gore et al., 2006; Gross et al., 2011).

3. Mechanics of a single collagen-fibril

3.1. Nano-micro transition by strain amplification

From the mechanical point of view fibrils can be considered as a cross-linked assembly of TC molecules. Tropocollagen molecules mainly receive their global stability by the covalent cross-linking initialized by specific lysine and hydroxysine residues (Kadler et al., 1996). In this regard, cross-links are assumed to be rigid (Linka and Itskov, 2015), which directly leads to the definition of the molecular stretch level λ_{tc} in terms of the fibril stretch λ_{fb} as

$$\overset{\boldsymbol{d}}{\lambda}_{tc} = \frac{\overset{\boldsymbol{d}}{\lambda}_{fb} - c}{1-c}. \tag{4}$$

This expression provides a bridge from the TC-level to the overlying fibril-level, where $\overset{\boldsymbol{d}}{(\bullet)}$ denotes the value of a physical quantity in the direction specified by a unit vector $\boldsymbol{d}$. Furthermore, it predicts a stiffer response of a fibril. This is due to the parameter c related to the cross-linking density. More specifically it is defined as a length ratio between the free molecular part and the cross-linked molecular part (for further details the interested reader is referred to Linka and Itskov, 2015). Henceforth, the parameter is chosen to be $c = 0.46$ in accordance with Sasaki and Odajima (1996).

3.2. Strain energy of a single collagen fibril

As mentioned in the previous section, the individual end-to-end length of every TC molecule uniquely assigns the molecule to the entropic or energetic state. Hence, the energy of a collagen fibril consisting of a certain amount of TCs (N_{tc}) can additively be decomposed into an entropic Ψ_s and an energetic Ψ_e energy part as

$$\Psi_{fb} = N_{tc}(\Psi_s + \Psi_e), \tag{5}$$