Journal of Biomechanics 46 (2013) 2497–2503

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
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Determining the contribution of glycosaminoglycans to tendon mechanical properties with a modified shear-lag model

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ARTICLE INFO

Article history:
Accepted 4 July 2013

Keywords:
Modeling
Fibril length
Extracellular matrix
Decorin
Proteoglycan

ABSTRACT

Tendon has a complex hierarchical structure composed of both a collagenous and a non-collagenous matrix. Despite several studies that have aimed to elucidate the mechanism of load transfer between matrix components, the roles of glycosaminoglycans (GAGs) remain controversial. Thus, this study investigated the elastic properties of tendon using a modified shear-lag model that accounts for the structure and non-linear mechanical response of the GAGs. Unlike prior shear-lag models that are solved either in two dimensions or in axially symmetric geometries, we present a closed-form analytical model for three-dimensional periodic lattices of fibrils linked by GAGs. Using this approach, we show that the non-linear mechanical response of the GAGs leads to a distinct toe region in the stress–strain response of the tendon. The critical strain of the toe region is shown to decrease inversely with fibril length. Furthermore, we identify a characteristic length scale, related to microstructural parameters (e.g. GAG spacing, stiffness, and geometry) over which load is transferred from the GAGs to the fibrils. We show that when the fibril lengths are significantly larger than this length scale, the mechanical properties of the tendon are relatively insensitive to deletion of GAGs. Our results provide a physical explanation for the insensitivity for the mechanical response of tendon to the deletion of GAGs in mature tendons, underscore the importance of fibril length in determining the elastic properties of the tendon, and are in excellent agreement with computationally intensive simulations.

1. Introduction

Tendon is composed of collagen molecules that assemble into collagen fibrils, which then bundle to form fibers and then fascicles. In addition, collagen fibrils are surrounded by a non-collagenous matrix, which consists of water and proteoglycans with their associated protein core and glycosaminoglycan (GAG) chains. Proteoglycan core proteins bind to collagen fibrils while their GAG chains span between the fibrils and can bind with other GAG chains or proteoglycan core proteins on the adjacent fibril (Scott and Hughes, 1986; Scott et al., 1981; Vesentini et al., 2005). The structural arrangement of tendon components suggests that both collagen fibrils and the non-collagenous matrix may play a role in stress transfer during uniaxial loading, though the actions of each component and their interactions are not fully established (Ansorge et al., 2012; Connizzo et al., 2013; Fessel and Snedeker, 2009; Lujan et al., 2007; Miller et al., 2012a, 2012b, 2012cProvenzano and Vanderby, 2006).

Several studies have examined tendon's collagenous matrix as a contributor to load transfer (Chen et al., 2009; Fratzl et al., 1998; Hansen et al., 2010; Robinson et al., 2004a; van der Rijt et al., 2006; Watanabe et al., 2007). In particular, some studies have shown that collagen fibrils are functionally continuous in adult tendon (Craig et al., 1989; Parry et al., 1978; Provenzano and Vanderby, 2006), suggesting that this may be the dominant mechanism of load transfer (Provenzano and Vanderby, 2006). Others have evaluated the tendon's non-collagenous matrix, indicating the GAGs as a potential mechanism for load transfer. The structure and relative movements of stained GAGs during mechanical relaxation tests suggest interfibrillar force transfer through a ratchet mechanism (Cribb and Scott, 1995; Watanabe et al., 2007, 2012). However, studies with enzymatic removal of GAGs have provided conflicting evidence concerning the role of GAGs in tendon mechanics (Fessel et al., 2012; Fessel and Snedeker, 2009, 2011; Lujan et al., 2007, 2009). Moreover, studies involving transgenic animals are controversial, with varied results in different species, tendons, and even regional variations within tendon (Connizzo et al., 2013; Dourte et al., 2012; Dunkman et al., 2013; Elliott et al., 2003; Robinson et al., 2005, 2004b).

Although collagen's contribution to tendon mechanical properties has been studied extensively (Gautieri et al., 2011, 2012;

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0021-9290/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jbiomech.2013.07.008

Heim et al., 2007; Svensson et al., 2010; van der Rijt et al., 2006), the role of GAGs remains unclear. Thus, several studies have used computational methods to model load transfer in the interfibrillar matrix (Fessel and Snedeker, 2011; Redaelli et al., 2003; Scott, 1992; Vesentini et al., 2005). Redaelli and colleagues evaluated the contribution of GAGs to stress transfer in tendons by introducing a piecewise linear GAG stiffness term in their micromechanical tendon fiber model (Redaelli et al., 2003). In particular, a multi-scale approach was utilized where the elastic properties of the GAGs were computed using molecular dynamics simulations in a 3D computational analysis to simulate stress–strain curves, as well as shear and tension profiles along the fibrils. The stress–strain curves show a clear nonlinearity at 2% strain. While this work provides important insights into load transfer and also considers variation of parameters, the range of fibril lengths considered (50–300 nm) is smaller than the typical length of fibrils (>1 mm) in adult tendon (Craig et al., 1989; Parry and Craig, 1984; Provenzano and Vanderby, 2006). In another study, Fessel and Snedeker developed a nice model containing a microstructure with randomly distributed fibrils of different diameters (Fessel and Snedeker, 2011). Results of their study showed that deletion of 80% of GAGs has a small influence on mechanical properties. However, no physical arguments were presented for the insensitivity of the response to GAG density, and their experiments showed smaller changes than their simulations.

One computationally efficient approach that has not yet been considered is a shear-lag model (SLM), which focuses on the transfer of tensile stress from matrix to fiber via interfacial stresses and can describe tendon mechanics at many strain levels (Cox, 1952; Okabe et al., 2001; Xia et al., 2002). Given that tendon is a fiber-reinforced composite that primarily experiences uniaxial loading, the SLM may be a useful tool in predicting mechanics. The mechanical response of tendon may depend on a number of factors, including non-linear GAG mechanical properties, GAG density, and fibril geometry (length, diameter, and spacing). Running simulations to analyze this large parameter space can be time consuming, particularly for large fibril lengths. Having an analytical model that can predict the dependence of these parameters can obviate the need for expensive 3D simulations and also provide physical insights into the role of different load bearing components.

Therefore, the objective of this work was to investigate the contribution of structural elements to overall tendon mechanics using a modified SLM that accounts for (1) the piece-wise linear stiffness characteristics of GAGs (Redaelli et al., 2003), (2) three-dimensional effects, such as variations in the magnitude of load transfer that arise when a given fibril has different numbers of neighbors, and (3) changes in the length scales over which load is transferred as GAGs are deleted. By including these features that are not considered in SLMs developed to date, we aim to elucidate the mechanisms by which GAGs contribute to tendon mechanical properties, and to identify other contributors (fibril length, modulus, diameter, area fraction, non-linear GAG mechanical properties, and GAG density) to tendon mechanics.

2. Model description and solution:

The SLM is implemented to describe the load transfer between fibrils and the GAGs. The model consists of uniform staggered fibrils interconnected with GAG cross-linkers that transfer the applied load via shear stress (Fig. 1). It is assumed that the fibrils are elastic with radius R_f (typically 75 nm) (Robinson et al., 2004a; Scott et al., 1981; Watanabe et al., 2012) and Young's modulus E_f (typically 1 GPa) (Svensson et al., 2012; van der Rijt et al., 2006). In addition, it is assumed that GAG tensile strain follows a bilinear

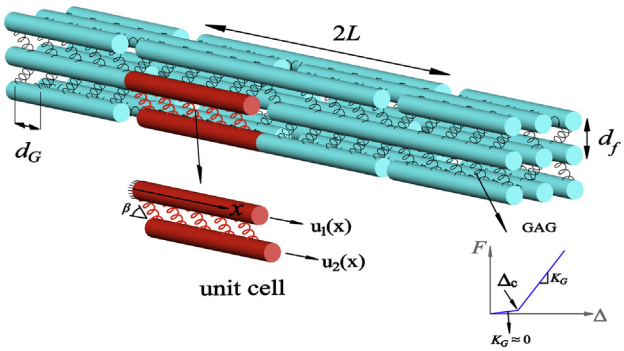

Fig. 1. The shear-lag model consists of fibrils (cyan) interconnected by GAGs (springs). A unit cell (red) with two neighboring fibrils is also shown. The force (F), elongation (Δ) behavior of the GAGs is also shown. Here, for $\Delta \leq \Delta_c$, the stiffness is negligible, while K_G denotes the stiffness when $\Delta > \Delta_c$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
List of symbols used in the modified shear-lag model.

L	Fibril half-length
E_f	Fibril Young's modulus
d_f	Fibril spacing
R_f	Fibril radius
K_G	GAG stiffness
d_G	GAG spacing
γ_G	GAG initial force-Free elongation
β	GAG-fibril angle
l_0	GAG- initial length
α	Fibril number of the closest-neighbors
ϕ	Fibril volume fraction

spring-like behavior, as observed in molecular simulations (Redaelli et al., 2003). This behavior arises from the highly coiled force-free state of GAGs. When GAGs are stretched, they uncoil easily as this involves breaking of weak van der Waals/hydrogen bonds. Molecular simulations show that the stiffness associated with breaking these bonds is $\gamma_G = 800\%$ (Redaelli et al., 2003). Above this value of critical strain, the energy needed to alter the covalent bonding energies (bond length and angles) increases significantly, leading to a substantial increase in stiffness, $K_G = 0.031$ N/m (Redaelli et al., 2003). Since this stiffness is about six orders of magnitude larger than the stiffness in the coiled regime, we assume that $K_G \approx 0$ when the strain is less than the critical strain for uncoiling γ_G. Each fibril is surrounded by α near-neighbor fibrils, where an α value of 4 and 6 corresponds to square and hexagonal distributions in the 3D space, respectively. The spacing between the fibrils and GAGs are denoted by d_f and d_G, respectively. Following Redaelli et al., $d_G = 68$ nm, corresponding to D-period length. Further, each GAG is attached to the fibril at an angle β (Fig. 1). The parameters that characterize the tendon microstructure are summarized in Table 1. A uniform staggered structure of fibrils with length $2L$ interconnected with GAGs with $\alpha = 4$ is shown in Fig. 1. The Cartesian coordinate system is placed at the center of each fibril such that the x-axis is oriented longitudinally along the fibril. A unit cell comprised of two neighbor fibrils (denoted by subscripts 1 and 2) with $0 \leq x \leq L$ is used to analyze load transfer between the fibrils and the GAGs.

The mechanical response is computed by imposing an overall strain on the structure and computing the resulting stress; specifically, we apply the boundary conditions $u_1(x = 0) = 0$ and $u_2(x = L) = \varepsilon L$. At the force-free ends of fibrils 1 and 2, the boundary conditions $\sigma_1(x = L) = E_f du_1(x)/dx = 0$ and $\sigma_2(x = 0) = E_f\, du_2(x)/dx = 0$ are applied. Since the GAGs with an initial length

Please cite this article as: Ahmadzadeh, H., et al., Determining the contribution of glycosaminoglycans to tendon mechanical properties with a modified shear-lag model. Journal of Biomechanics (2013), http://dx.doi.org/10.1016/j.jbiomech.2013.07.008