Possible age-related changes in the mechanical properties of tendons of mimecan-deficient mice

K.L. Goh[1,2,#]

[1]School of Mechanical & Systems Engineering, Newcastle University, UK, [2] Composite Mechanics Laboratory, NUIS, Singapore

[#]Email: kheng-lim.goh@ncl.ac.uk

Abstract. Mimecan, also known as osteoglycin, belongs to the family of small leucine-rich proteoglycans (SLRPs). To a large extent, it is often assumed that mimecan is expected to be associated with the development and maintenance of the structure of the collagen fibril network in the tissue similar to the other SLRPs. Since collagen fibrils are responsible for the reinforcement of the tissue, it is believed that the absence of mimecan could affect the tissue mechanical properties. Building on the work of previous results on changes in structure and mechanical properties, this study tested the tendons from mimecan-deficient mice to evaluate the tissue strength, strain at maximum stress, modulus of elasticity and strain energy density to fracture in mimecan-deficient mice, in the presence of ageing, in order to gain further insights into how absence of mimecan affects the structure-function relationship of connective tissue.

Introduction

SLRPs are components of the extracellular matrix (ECM) of connective tissues, featuring leucine-rich repeats and a cysteine-rich cluster in the N-terminal region which have highly conserved spacing [1]. SLRPs are associated with regulatory roles such as the development and maintenance of the collageneous-structure of the tissue [1]. Mimecan, also known as osteoglycin, belongs to the SLRP family [2]. Mimecan proteoglycans (PGs) are attached with keratan sulfate (KS), which is a member of the glycosaminoglycan (GAG) polysaccharides; GAG sulfation gives the mimecan PG a net negative charge and facilitate water-binding [3]. Mimecan, lumican [4] and keratocan [5] are the three major KS-containing PGs in the cornea [6].
The role of these molecules in the tissue structure has been investigated using mimecan-deficient mice [2,7]. Some studies have suggest that mimecan has a role in regulating the collagen fibrollogenesis [2]. For instance, in the cornea and skin, appreciable alterations in collagen fibril size and morphology can occur in 1 month-old (Mo) mimecan-deficient mice [2]; the mechanical (structure-related) strength of skin in the mutant mice is significantly lower than that from wild types [2]. Yet, these mutant mice show no apparent abnormality at the macroscopic level, e.g. no changes in clarity and thickness of the cornea [2]. More interestingly, in older (4 Mo) mice, the cornea of the mutant and wild-type mice exhibits no difference in fibril diameter and interfibrillar spacing [8]. Previous studies have shown how structure-function relationship in connective tissues may be established in the presence of ageing [9,10]. The purpose of the present study was to assess the effect of the absence of mimecan on the mechanical properties of connective tissues in the experimental model of knockout mouse in the presence of ageing. The insights gained in previous studies would be applied to explain the possible role of mimecan in the modification of the structure-function relationship.

Methods

The author was provided with tendons from the tail of C57BL6 mimecan-deficient mice and wild types, courtesy of Prof Gary W Conrad (Division of Biology, Kansas State University, Manhattan, KS) from different age classes: 1-2 Mo (x3), 4-5 Mo (x3) and 7-9 Mo(x5). The procedure for the reproduction of these mimecan-deficient mice has been described elsewhere [2]. Tendons (500 micron thick) were removed from the tail by sliding them out along the tail. For each individual mouse, twelve segments (7 mm long) from randomly selected tendons were prepared. The procedure for micromechanical testing of soft tissues with micrometer dimensions has been described in details elsewhere [9–13]. Here, a small scale horizontal tensile test frame device was used to stretch each segment at 0.067 mm/s from a slightly slackened state until it was ruptured. Throughout the experiment each sample was hydrated by submerging in phosphate buffer saline (pH 7.2) solution in a petri-dish. Data of load versus extension of each specimen acquired from the tendon was evaluated to obtain stress versus strain data to determine the material (mechanical) properties. The stress-strain curves featured a S-shape profile typical of collagen fascicles described elsewhere [9–13]. The tendon strength (σ_U) was identified with the maximum stress. To determine the stiffness (E) at each age class, a fifth order polynomial equation was used to fit the stress-strain data points (from each sample) from $\varepsilon = 0$ to the strain (ε_U) at σ_U using a least-square method [9–13]. The fracture toughness (u_F) was determined from the area under the stress-strain curve from the origin until the end point, using the trapezoidal rule.

Results & Discussion

With regards to the effects from the interaction of mimecan and age, all mechanical properties yielded no significant differences ($p > 0.05$). With regards to the main effects of age on the mechanical properties, while age has no effect on the E ($p > 0.05$), significant differences are observed for σ_U ($p < 0.01$), ε_U ($p < 0.005$) and u_F ($p = 0.001$), respectively. In wild types, the magnitudes of the σ_U, ε_U and u_F are small but as ageing

progresses, the magnitudes of these tensile properties increase, which are entirely consistent with results reported elsewhere [9,10]. Interestingly, a similar trend is also exhibited by the tendons of mimecan-deficient mice. With regards to the possible influence of mimecan on the mechanical properties, it is shown that the absence of mimecan has no effect on the E, σ_U and ε_U of the tail tendons from all the age classes ($p > 0.05$). However, significant differences are observed for the u_F ($p = 0.038$) of tendons, between the wild types and mimecan-null mice—further analysis (i.e. t test) reveals that the differences occur in the 7-9 Mo age class.

Do the structure of the fibrils, namely the fibril volume fraction, contribute to a linear (positive) effect on the tissue strength, in accordance to the rule of mixture for fibre reinforced composite [9]? So far, there is little attempt at establishing such a structure-function relationship. As mentioned in the introduction section, Tasheva et al. reported that the mechanical (structural) strength of the skin of (1 Mo) mimecan-deficient mice (5.9 N) is lower than that of (1 Mo) wild-type (7.9 N) (NB: Tasheva et al. reported no significant differences in the thickness of the skin specimens) [2]. At the microscopic level, structural analysis of the skin (as well as cornea) of these (1 Mo) mice reveals that the diameter of collagen fibrils from the mimecan-null mice are significantly larger than those from wild-type [2]. However, it is not clear if this translates to a smaller fibril volume fraction. Qualitative examination of electron micrographs suggests that there is no difference in the looseness of the packing of fibrils in both mutant and wild type. In contrast, the fibrils in mimecan-deficient mice are observed to be more loosely packed than those in wild types [2]. If this translates to a smaller fibril volume fraction, then the tensile strength of these cornea must be lower than in wild types. Unfortunately, no results on the mechanical strength of the cornea have been reported. Curiously too, cornea from the (4 Mo) mutant and wild-type mice reveals no appreciable differences with regards to these structural features [8]. Unfortunately, it is not clear if that implicates that the mechanical strengths of the cornea from the mutant and wild type are also similar. In the case of tendons from the tail, previous report suggests that the structure-function relationship of the u_F with respect to fibril diameter is complicated by the competition between small and large fibrils. But exactly how do the small and large fibrils compete to reduce the u_F in mimecan-deficient mice fibril diameter remains to be investigated. Future work to be carried out include electron microscopy, to analyse the fibril thickness and morphology that can lend support to the analysis of the mechanical properties of the tendon.

Over the last 15 years, the significance of the role of mimecan is gradually unravelling. Recently, it is shown that mimecan (as well as some ECM glycoproteins) may be involved in cardiac remodelling of the myocardium [14]. Aortic stenosis is associated with increase in the expression of SLRP osteoglycin [15]. Mimecan may also be involved in the pathogenesis of atherosclerosis by regulating the proliferation, apoptosis and migration of vascular smooth muscle cells [16]. More recently, it is shown that mimecan core protein (not glycosaminoglycan) could cross-link with collagen in the presence of UVA—which is an important contributor to premature ageing of the skin—and riboflavin [17].

References

1 Chen S & Birk DE (2013) The regulatory roles of small leucine-rich proteoglycans in extracellular matrix assembly. *FEBS J.* **280**, 2120–2137.

2 Tasheva ES, Koester A, Paulsen AQ, Garrett AS, Boyle DL, Davidson HJ, Song M, Fox N & Conrad GW (2002) Mimecan/osteoglycin-deficient mice have collagen fibril abnormalities. *Mol. Vis.* **8**, 407–415.

3 Casroro JA, Berrelheim AA & Berrelheim FA (1988) Water gradients across bovine cornea. *Investig. Ophthalmol. Vis. Sci.* **29**, 963–968.

4 Blochberger TC, Vergness J-P, Hempel J & Hassell JR (1992) cDNA to chick lumican (corneal keratan sulfate proteoglycan) reveals homology to the small interstitial proteoglycn gene family and expression in muscle and intestine. *J. Biol. Chem.* **267**, 347–352.

5 Corpuz LM, Funderburgh JL, Funderburgh ML, Bottomley GS, Prakash S & Conrad GW (1996) Molecular cloning and tissue distribution of keratocan. *J. Biol. Chem.* **271**, 9759–9763.

6 Funderburgh JL, Corpuz LM, Roth MR, Funderburgh ML, Tasheva ES & Conrad GW (1997) Mimecan, the 25-kDa corneal keratan sulfate proteoglycan, Is a product of the gene producing osteoglycin. *J. Biol. Chem.* **272**, 28089–28095.

7 Tasheva ES, Ke A, Deng Y, Jun C, Takemoto LJ, Koester A & Conrad GW (2004) Differentially expressed genes in the lens of mimecan-null mice. *Mol. Vis.* **10**, 403–416.

8 Beecher N, Carlson C, Allen BR, Kipchumba R, Conrad GW, Meek KM & Quantock AJ (2005) An x-ray diffraction study of corneal structure in mimecan-deficient mice. *Investig. Ophthalmol. Vis. Sci.* **46**, 4046–4049.

9 Goh KL, Holmes DF, Lu HY, Richardson S, Kadler KE, Purslow PP & Wess TJ (2008) Ageing changes in the tensile properties of tendons : influence of collagen fibril volume. *J. Biomech. Eng.* **130**, 21011.

10 Goh KL, Holmes DF, Lu Y, Purslow PP, Kadler KE, Bechet D & Wess TJ (2012) Bimodal collagen fibril diameter distributions direct age-related variations in tendon resilience and resistance to rupture. *J. Appl. Physiol.* **113**, 878–888.

11 Goh KL, Chen SY & Liao K (2014) A thermomechanical framework for reconciling the effects of ultraviolet radiation exposure time and wavelength on connective tissue elasticity. *Biomech. Model. Mechanobiol.*

12 Goh KL, Chen Y, Chou SM, Listrat A, Bechet D & Wess TJ (2010) Effects of frozen storage temperature on the elasticity of tendons from a small murine model. *Animal* **4**, 1–5.

13 Yeo YL, Goh KL, Kin L, Wang H, Listrat A & Bechet D (2011) *Structure-property relationship of burn collagen reinforcing musculo-skeletal tissues*.

14 Iozzo R V & Schaefer L (2015) Proteoglycan form and function: A comprehensive nomenclature of proteoglycans. *Matrix Biol.* **42**, 11–55.

15 Rienks M, Papageorgiou A-P, Frangogiannies NG & Heymans S (2014) Myocardial extracellular matrix: an ever-changing and diverse entity. *Circ. Res.* **114**, 860–871.

16 Zhang H-J, Wang J, Liu H-F, Zhang X-N, Zhan M & Chen FL (2015) Overexpression of mimecan in human aortic smooth muscle cells inhibits cell proliferation and enhances apoptosis and migration. *Exp. Ther. Med.* **10**, 187–192.

17 Zhang Y, Conrad AH & Conrad GW (2011) Effects of ultraviolet-A and riboflavin on the interaction of collagen and proteoglycans during corneal cross-linking. *J. Biol. Chem.* **286**, 13011–13022.