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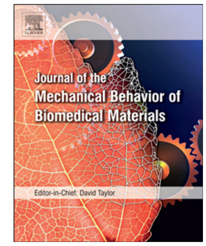

Research Paper

Structure–mechanics relationships in mineralized tendons

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ABSTRACT

In this paper, we review the hierarchical structure and the resulting elastic properties of mineralized tendons as obtained by various multiscale experimental and computational methods spanning from nano- to macroscale. The mechanical properties of mineralized collagen fibres are important to understand the mechanics of hard tissues constituted by complex arrangements of these fibres, like in human lamellar bone. The uniaxial mineralized collagen fibre array naturally occurring in avian tendons is a well studied model tissue for investigating various stages of tissue mineralization and the corresponding elastic properties. Some avian tendons mineralize with maturation, which results in a graded structure containing two zones of distinct morphology, circumferential and interstitial. These zones exhibit different amounts of mineral, collagen, pores and a different mineral distribution between collagen fibrillar and extrafibrillar space that lead to distinct elastic properties. Mineralized tendon cells have two phenotypes: elongated tenocytes placed between fibres in the circumferential zone and cuboidal cells with lower aspect ratios in the interstitial zone. Interestingly some regions of avian tendons seem to be predestined to mineralization, which is exhibited as specific collagen cross-linking patterns as well as distribution of minor tendon constituents (like proteoglycans) and loss of collagen crimp. Results of investigations in naturally mineralizing avian tendons may be useful in understanding the pathological mineralization occurring in some human tendons.

1. Introduction

Mineralized collagen fibres are mainly associated with bone tissue, but are actually constituting several tissues, one of which is the mineralized avian tendon. Mineralized tendon has been widely investigated in terms of composition and structure as an interesting tissue per se. Due to the similar composition to bone and the relative simplicity of its structure, mineralized avian tendons tissue is often used as a structural model of bone, but its structure–function relationships are not yet completely elucidated. In this paper, we review the current state of knowledge on structure and mechanics of mineralized tendons. First, we describe their hierarchical structure, composition and cell populations. Further, we report the mechanical properties of this tissue at multiple length scales, as assessed by experimental and computational modelling techniques.

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In the literature, a distinction is made between primary and secondary mineralization. Primary mineralization is the process of infiltrating soft matrix with calcium and phosphate ions and then precipitating them in this matrix within 5–10 days. This process provides up to 50% of the maximal mineralization level in mature bone (Boivin et al., 2009). Secondary mineralization is the subsequent process of slow, gradual maturation of the mineral component of tissue that may take up to 30 months in humans. This second process includes not only an increase in mineral content but also an increase in the number of mineral crystals, their size and crystallinity (Boivin et al., 2009). The mechanisms of stiffening of avian tendons with the progress of mineralization are not entirely clear. They may involve both primary and secondary mineralization, and we consequently choose not to distinguish between them and refer to the process as "mineralization". The mineralization of tissues has been studied extensively for many years now and the understanding of the biophysics of this complex process increases with time, even though some details remain obscure.

The term mineral used in the literature in the context of bone or other mineralized collagenous tissues is a simplification of the inorganic phase. The mineral phase of bone consists of calcium phosphate, often referred to as non-stoichiometric hydroxyapatite or dahllite. The content of calcium in bone mineral is lower than in a synthetic, highly crystalline hydroxyapatites, which is reflected in its physical properties (Glimcher, 2006). Calcium is partially replaced with sodium ions and the phase contains significant amounts of carbonate and hydrogen phosphate groups (Glimcher, 2006). The minerals are also to some extent amorphous and contain other ingredients such as citrate (Davies et al., 2014). At this scale, bone mineral forms plate-like nanocrystals with thickness of 1.5–4.5 nm as measured by transmission electron microscopy, small-angle X-ray scattering and atomic force microscopy (Fratzl and Weinkamer, 2007; Fratzl et al., 2004; Rubin et al., 2003; Rubin and Jasiuk, 2005; Eppell et al., 2001). The mineral platelets placed within collagen fibrils seem to be arranged mostly parallel to each other and along the fibrils long axis. The mineral can be described as poorly crystalline hydroxyapatite or poorly ordered nanocrystals of hydroxyapatite. A certain fraction of mineral may be placed in the extrafibrillar space. The fraction of mineral deposited within the fibrils is considerably debated in the literature.

The early stages of bone mineralization are not easily observed, therefore some physiologically mineralizing avian leg tendons have served as a vertebrate mineralized tissue model. The advantage of such a model is the fact that multiple sequential mineralization stages can be observed in one sample at the same time (Landis and Silver, 2002; Landis, 1986). Some tendons of avians mineralize over time creating a tissue similar in composition to bone, but with retained uniaxial arrangement of collagen fibres as in tendons. It is not completely clear why some avian tendons mineralize, while others do not (Landis and Silver, 2002). For example, not all of the tendons in turkeys mineralize and some avians do not have any mineralizing tendons. The mineralization processes in avian tendons and other pathologically mineralizing tissues of other species are likely complex and may include contributions of various biological and biophysical factors (Landis and Silver, 2002).

More recently, mineralized avian tendon tissue has been used as a simplified mechanical model of bone (Gupta et al., 2004; Lees and Page, 1992). Because of its approximately uniaxial structure, its mechanical properties are easier to access as a major source of complexity, heterogeneous fibre orientation is excluded when compared to bone tissue. Mineralized turkey leg tendon (MTLT) is the tissue used most frequently as a model tissue. Naturally mineralizing tendons can also serve as models for the pathological mineralization occurring in human tendons called calcific tendinitis (Riley, 2010). This condition causes pain and reduced mobility of tendons such as rotator cuff, Achilles, biceps brachii, extensor pollicus longus or quadriceps tendons (Riley, 2010; O'Brien et al., 2012). Some authors claim that mineralization in tendons is a result of ectopic bone formation (O'Brien et al., 2012), others suggest that the process of mineralization may be cell-mediated (Rui et al., 2011; Fenwick and Harrall, 2002) or a result of disrupted differentiation of tendon-derived stem-like cells (Clegg et al., 2007). The details of these processes remain obscure.

In this review, we describe the current state of knowledge of the hierarchical structure of mineralized tendons in terms of mineralization, collagen organization, collagen cross-links, cellularity and porosity, as well as their elastic properties across the length scales. Both experimental and computational modelling contributions are considered.

2. Structure

Domestic turkey (*Meleagris galopavo*) leg tendons are studied most frequently as a model of mineralization or/and mechanics of mineralized collagen fibres (Prostak and Lees, 1996; Knott et al., 1997; Lees et al., 1994; Landis et al., 1996; Silver et al., 2001; Traub et al., 1989; Landis and Silver, 2002; Siperko and Landis, 2001; Spiesz et al., 2012b). Also tendons of domestic chicken (*Gallus domesticus*) have been studied (Agabalyan et al., 2013; Ono and Nemoto, 2005). The investigated tendon types include Achilles (gastrocnemius) and/or digital flexor tendons. To our knowledge, differences between different tendon types have not been addressed specifically in the literature.

2.1. *Mineralization process*

Avian leg tendons mineralize naturally and the mineralization is partial, some non-mineralized tendon tissue persists even though the tendons are sometimes referred to as fully mineralized, which means that the maximum mineralization level is thought to be reached at some location. Mineralization starts at 10–17 week of animal life (Landis, 1986; Knott et al., 1997; Silver et al., 2003; Liu and Kuboki, 1996) and tendons are considered fully mineralized after 24 weeks.

The origin of the mineralization process has been shown to be the matrix between collagen fibres and possibly also between collagenous fibre bundles. The so-called vesicles (20–200 nm in diameter) (Landis and Silver, 2002) seem to be located in-between adjacent collagen fibres in the longitudinal direction. In this sense, vesicles are similar to interfascicular matrix in tendons (Thorpe et al., 2012, 2015; Spiesz et al., 2015). The vesicles occupy spaces between fibres rather than fibre bundles, which is one level of tendon hierarchy lower than the

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