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Matrix Biology 25 (2006) 71 – 84
MATRIX BIOLOGY
www.elsevier.com/locate/matbio

Collagen fibril morphology and organization: Implications for force transmission in ligament and tendon

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Received 1 June 2005; received in revised form 19 September 2005; accepted 20 September 2005

Abstract

Connective tissue mechanical behavior is primarily determined by the composition and organization of collagen. In ligaments and tendons, type I collagen is the principal structural element of the extracellular matrix, which acts to transmit force between bones or bone and muscle, respectively. Therefore, characterization of collagen fibril morphology and organization in fetal and skeletally mature animals is essential to understanding how tissues develop and obtain their mechanical attributes. In this study, tendons and ligaments from fetal rat, bovine, and feline, and mature rat were examined with scanning electron microscopy. At early fetal developmental stages, collagen fibrils show fibril overlap and interweaving, apparent fibril ends, and numerous bifurcating/fusing fibrils. Late in fetal development, collagen fibril ends are still present and fibril bundles (fibers) are clearly visible. Examination of collagen fibrils from skeletally mature tissues, reveals highly organized regions but still include fibril interweaving, and regions that are more randomly organized. Fibril bifurcations/fusions are still present in mature tissues but are less numerous than in fetal tissue. To address the continuity of fibrils in mature tissues, fibrils were examined in individual micrographs and consecutive overlaid micrographs. Extensive microscopic analysis of mature tendons and ligaments detected no fibril ends. These data strongly suggest that fibrils in mature ligament and tendon are either continuous or functionally continuous. Based upon this information and published data, we conclude that force within these tissues is directly transferred through collagen fibrils and not through an interfibrillar coupling, such as a proteoglycan bridge.

Keywords: Extracellular matrix; Structure-function; Scanning electron microscopy; Three-dimensional matrix organization; Cell–matrix interaction

1. Introduction

Collagens, the primary structural elements of the extracellular matrix, are the most abundant proteins in tissues such as ligament, tendon, cartilage, bone, cornea, and skin. Type I collagen assembles, via collagen molecules, into collagen fibrils which are long filamentous structures which aggregate to form collagen fibers (Nimni and Harkness, 1988). In vivo, type I collagen fibrillogenesis is a multi-step process involving intracellular and extracellular compartments defined by the fibroblast (Birk and Trelstad, 1984, 1986; Birk et al., 1989; Canty et al., 2004). Collagen fibril segments then form intermediate structures that assemble into collagen fibrils and undergo post-depositional growth during embryonic development (Birk et al., 1995, 1989, 1997). For instance, in tendons from embryonic chickens, fibrils substantially increase in length between 14 and 17 days (Birk et al., 1995). After 17 days of embryonic development, fibril length dramatically increases (Birk et al., 1996, 1995). By 18 days of embryonic development, Birk et al. had great difficulty identifying both ends of the collagen fibrils when examining the tendon over the same tissue length in which both ends of fibrils were easily identifiable in 14 day embryos; further illustrating the rapid and substantial fibril lengthening at this stage of development (Birk et al., 1997). This increase in fibril length during embryonic development may be the result of lateral association or fusion between collagen fibrils, producing longer and larger diameter fibrils (Birk et al., 1997), or by tip-to-tip fusions of collagen fibrils to produce longer fibrils (Graham et al., 2000), or both. Exact mechanisms of fibril lengthening during development require further study.

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doi:10.1016/j.matbio.2005.09.005

During collagen fibrillogenesis, proteoglycans play a large role in guiding and stabilizing collagen fibril formation and maturation. Several studies have shown that decorin, lumican, and fibromodulin, members of the small leucine-rich proteoglycan (SLRP) family, play a role in regulating collagen fibril organization and maturation (Birk et al., 1995; Chakravarti et al., 1998; Danielson et al., 1997; Ezura et al., 2000; Graham et al., 2000; Jepsen et al., 2002; Keene et al., 2000; Scott, 1996; Scott et al., 1981; Svensson et al., 1999; Vogel and Trotter, 1987). In relation to type I collagen, decorin is located along the fibril shaft and is attached to the fibril surface via noncovalent bonding (Scott and Orford, 1981) with its glycosaminoglycan (GAG) chain extending laterally from the fibril, possibly maintaining hydration and interfibrillar spacing (Scott, 1988), and is absent at collagen fibril ends where tip-to-tip fusion can occur (Graham et al., 2000). In addition to decorin, both fibromodulin and lumican have also been implicated in regulating collagen fibrillogenesis. Like decorin, these SLRPs are envisioned as having a horseshoe shaped core protein in which the concave surface laterally associates with collagen and the GAG chain extends laterally away from the fibril (Scott, 1996; Weber et al., 1996). When collagen fibrils from mice deficient in fibromodulin and/or lumican are examined, they exhibit abnormal development in terms of diameter and shape (Chakravarti et al., 1998; Ezura et al., 2000; Jepsen et al., 2002; Keene et al., 2000; Svensson et al., 1999). Therefore, in vivo SLRPs, such as decorin, fibromodulin, and lumican, strongly regulate multiple aspects of collagen fibrillogenesis and consequent integrity.

As described above, during embryonic development collagen fibrils are known to be discontinuous units, which profoundly increase in length as development progresses. Less information is known about the length of the collagen fibril at birth, through the skeletally immature growth phase, and after skeletal maturity has been reached. It is known that material properties, such as ultimate stress, of the collagenous extracellular matrix (ECM) increase more than three fold from skeletally immature animals to more developed or skeletally mature animals (Beredjiklian et al., 2003; McBride et al., 1988; Provenzano et al., 2002a; Woo et al., 1986), implying a substantial change to the extracellular matrix must occur as the animal matures; as indicated via continuous collagen fibers in chick tendon during late embryonic development and shortly after birth (McBride et al., 1985). To explain these changes, in addition to known changes associated with cross-linking and fiber morphology, experiments have been conducted and hypotheses discussed regarding the length of collagen fibrils within collagen fibers in mature animals, and the organization and composition of the ECM, particularly the proteoglycan–collagen interaction, in skeletally mature animals. One such study by Craig et al. (1989) theoretically illustrates that collagen fibril length increases from birth to maturity with mature fibrils reaching lengths greater than 10 mm. Yet, controversy still exists regarding the length and continuity of collagen fibrils in skeletally mature tendons and ligaments.

Many authors assume or conclude that the strong majority of collagen fibrils are long (millimeters in length) and either span the length of the ligament or tendon or are long enough to be considered functionally continuous (Birk et al., 1995, 1997; Craig et al., 1989; Graham et al., 2000; Holmes et al., 1998; Parry and Craig, 1984). For instance, utilizing transmission electron microscope to examine fibril length in mature tendon, Trotter and Wofsy (1989) examined 5639 tendon fibrils (over 4.26 mm of fibril length) and found two ends from small fibrils, while Parry and Craig (1984) examined 1000 fibrils and Craig et al. (1989) 1368 fibrils and found no ends. In contrast, other authors suggest that the majority of fibrils in mature mammalian tendon and/or ligament are short discontinuous fibrils (Caprise et al., 2001; Dahners et al., 2000; Derwin and Soslowsky, 1999; Derwin et al., 2001; Mosler et al., 1985; Nemetschek et al., 1983; Raspanti et al., 2002; Redaelli et al., 2003; Robinson et al., 2004). Accompanying the hypothesis of short discontinuous fibrils is the hypothesis that force must be transferred between fibrils through a mechanical coupling (Mosler et al., 1985; Nemetschek et al., 1983), typically SLRPs (Caprise et al., 2001; Dahners et al., 2000; Derwin and Soslowsky, 1999; Derwin et al., 2001, Redaelli et al., 2003; Robinson et al., 2004). Although fibril length was not addressed, in mammalian tendon, Cribb and Scott (1995) proposed the concept of proteoglycan bridges playing a role in transmitting and resisting tensile stress. More recent data, however, have not strongly supported the concept of a force transmitting proteoglycan bridge. Dahners et al. (2000) hypothesized that "decorin–fibronectin binding is an important link in interfibrillar bonding" and applied NKISK (an agent that inhibits binding of decorin to fibronectin) to isolate intact fibrils from mature rat ligament and tendon. Only ten intact small diameter fibrils ligament and sixteen possibly intact small diameter tendonous fibrils were obtained. That is, the vast majority of fibrils were either long or not isolated by this method, and administration of NKISK had no detrimental effect on tissue strength or stiffness (Caprise et al., 2001). Furthermore, work in mov13 mice, reveals no significant difference in maximum load, stress, stiffness, modulus, or total collagen between tendon fascicles from mov13 and control mice, even though the ratio of decorin to collagen is decreased in the mov13 mice (Derwin and Soslowsky, 1999; Derwin et al., 2001). In accordance with the above studies, tendons from decorin knockout mice reveal no significant difference in maximum load, stress, stiffness, or modulus between age matched controls (Forslund et al., 2002, Gimbel et al., 2002, Lin et al., 2002, Robinson et al., 2004) and the adding of decorin to self-assembled collagen fibers does not significantly increase the ultimate stress of the tissue, but may help facilitate slippage between fibrils (Pins et al., 1997). Additionally, mice deficient in both fibromodulin and lumican show an appreciable decrease in the modulus (Jepsen et al., 2002). Yet, due to the extremely abnormal cross-sectional morphology and distribution of collagen fibrils, resulting from the combined lumican/fibromodulin deficiency during development, it would appear that changes in collagen cause the