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Potential of collagen cross-linking therapies to mediate tendon mechanical properties

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Collagen cross-links are fundamental to the mechanical integrity of tendon, with orderly and progressive enzymatic cross-linking being central to healthy development and injury repair. However, the nonenzymatic cross-links that form as we age are associated with increased tendon brittleness, diminished mechanical resistance to injury, and impaired matrix remodeling. Collagen cross-linking thus sits at the center of tendon structure and function, with important implications to age, disease, injury, and therapy. The current review touches on these aspects from the perspective of their potential relevance to the shoulder surgeon. We first introduce the most well-characterized endogenous collagen cross-linkers that enable fibrillogenesis in development and healing. We also discuss the glycation-mediated cross-links that are implicated in age- and diabetes-related tendon frailty and summarize work toward therapies against these disadvantageous cross-links. Conversely, we discuss the introduction of exogenous collagen cross-links to augment the mechanical properties of collagen-based implants or native tendon tissue. We conclude with a summary of our early results using exogenous collagen cross-linkers to prevent tendon tear enlargement and eventual failure in an in vitro model of partial tendon tear.

Level of evidence: Review Article.

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Whether in the early stages of embryonic tendon development or in the late stages of rotator cuff tendon tear, collagen cross-links play a key role in tissue function and matrix modeling. Despite collagen cross-linking being a central factor in tendon strength, stiffness, and toughness, therapeutic approaches attempting to modulate collagen cross-links to improve tendon healing or ameliorate tendon disorders remain largely unexplored. We consider such approaches to represent a large and mostly untapped potential that warrants increased scientific attention. The current review thus seeks to highlight the integral role of collagen cross-linking in tendon structure-function and how we may possibly alter the molecular structure of tendon for therapeutic gain.

Tendons not only enable efficient transfer of force from muscle to bone but also provide a small but important degree of viscoelasticity. This complex functionality is enabled by a highly organized hierarchic structure built of longitudinally aligned collagen molecules that self-assemble into fibrils. These fibrils are embedded in a proteoglycan-rich matrix and form larger collagen fibers, which in turn form fascicles, and finally, multiple fascicles to compose a whole tendon.[33] Although collagen is clearly the main load-bearing

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molecule, the mechanical competence of individual fibrils heavily depends on the enzyme lysyl oxidase, which regulates the robust formation of stable intermolecular collagen cross-links during maturation.[5] The absence of these head-to-tail chemical bonds drastically diminishes collagen fibril strength and whole tissue function.[29,50] We later discuss the critical importance of lysyl-oxidase mediated cross-links and how they transform self-assembled collagen molecules into mechanically robust collagen fibrils during maturation.

In stark contrast to the enzymatic cross-links involved in normal fibrillogenesis, mechanically disadvantageous covalent bonds between collagens can form as collagens react with sugars, a process called glycation. Subsequent oxidation of these reactive products leads to the formation of advanced glycation end-products (AGEs), some of which are cross-links. The onset of AGE cross-links is well characterized in normal aging and in diabetes, and AGE cross-links have been associated with increased fibrillar stiffness, loss of tissue toughness, impaired matrix remodeling, and the chronic inability of tendon to cope with microdamage.[2,3] We review here in some depth how AGEs accumulate in aging and in diabetes, as well as the possible use of cross-link–breaking therapies against these functionally disadvantageous matrix components.

We also discuss the therapeutic potential of large-molecule, transfibrillar cross-links. We consider the known range of potential endogenous macromolecules that may bridge neighboring collagen fibrils to enable tendon function. Theoretic studies by our laboratory and others have demonstrated that transfibrillar cross-links have the potential to substantially affect tissue level mechanics,[20,52,72] and we review here the relatively few experimental studies that have explored the use of such cross-links to augment tendon function.[10,14,40]

We conclude this review with a summary of research related to use of exogenous collagen cross-linking to augment tendon mechanics (the intentional introduction of cross-links). Exogenous cross-linking has emerged as a promising approach in biomaterial science to modulate the mechanical properties of native tissues and tissue-engineered implants, with a judicious selection of collagen cross-linkers potentially conferring superior mechanical traits. We summarize some of our recent work characterizing candidate cross-linking agents for their mechanical effects on tendon and briefly discuss the ability of one cross-linker, Genipin, to arrest tear propagation in a model of acute partial-tendon tear. We then discuss the necessary balancing of mechanical considerations against biologic considerations and identify challenges that must be faced in translating collagen cross-linking to a viable clinical treatment.

Cross-linking in development and maturation

Tendons become more stiff as they develop and mature.[27,68] The major contributors to increased structural stiffness are larger tendon cross-section, increased collagen content, and alterations in the structural arrangement of collagen fibrils.[27,56] The relative density, total number, and morphologies of collagen fibrils are thus correlated to tendon function, but the mechanical properties of the individual collagen fibrils largely drive whole tendon behavior. This is regulated by proper formation of covalent collagen cross-links. These covalent cross-links are formed head-to-tail at the overlapping ends of adjacent collagen molecules that adopt a quarter-stagger configuration (Fig. 1). The major type I collagen cross-links in tendon are driven by the enzyme lysyl oxidase, which specifically acts on lysine or hydroxylysine in the telopeptide region of the collagen molecule and results in a divalent, immature cross-link with an opposing amino acid in the triple-helical region.[47] Spontaneously, these immature cross-links convert into more stable trivalent cross-links that increase collagen interconnectivity, fibril stability, and whole tendon mechanical integrity.[5,19]

Simple biochemical correlations of native cross-link content with tendon mechanical properties are rather weak,[7,13,28,67] reflecting the likely confounding influence of other dominant structural or compositional factors.[56] The essential functional role of cross-linking in collagen fibril stability and whole tissue integrity, however, is clearly demonstrated in the severely compromised connective tissues of animals subjected to dietary inhibition of lysyl oxidase, which results in collagen fibrils and tendons with reduced strength.[29,50] The importance of cross-links to fibril integrity has been indicated theoretically[70] and demonstrated experimentally[45,50] by balancing molecular slip and stretch under load. The involvement of cross-linking in preventing molecular slippage and resultant fibrillar damage can also be inferred from the decreased thermal stability of tendons that is known to take place after submaximal tissue overload.[74] Given that lysyl oxidase mediated cross-links are so essential to the proper development of fibril structure and mechanical integrity, these are perhaps the best characterized collagen cross-linkers. The following section discusses less well-characterized macromolecular cross-links that may hold potential in therapeutic modulation of tendon mechanics.

Transfibrillar cross-linking molecules: early investigations into fibronectin- and proteoglycan-mediated fibril load sharing

In addition to the enzyme-mediated covalent bonds between collagen molecules, various extracellular macromolecules have been hypothesized to have important structural interactions with type I collagen and thus mediate tendon mechanical function. More specifically, macromolecules have been proposed to cross-link between adjacent collagen fibrils, thus acting to stabilize the collagen network. These interactions have been studied in cartilage, where potentially important binding between collagen types II and IX have been proposed.[18] In tendon, analogous interactions of