THE TENDON-TO-BONE ATTACHMENT

Unification through disarray

High-resolution imaging, composition analysis and mechanical testing reveal a disordered transitional material within the Achilles tendon-to-bone attachment, structured as a fibrous network to enable force transfer and maximize structural integrity.

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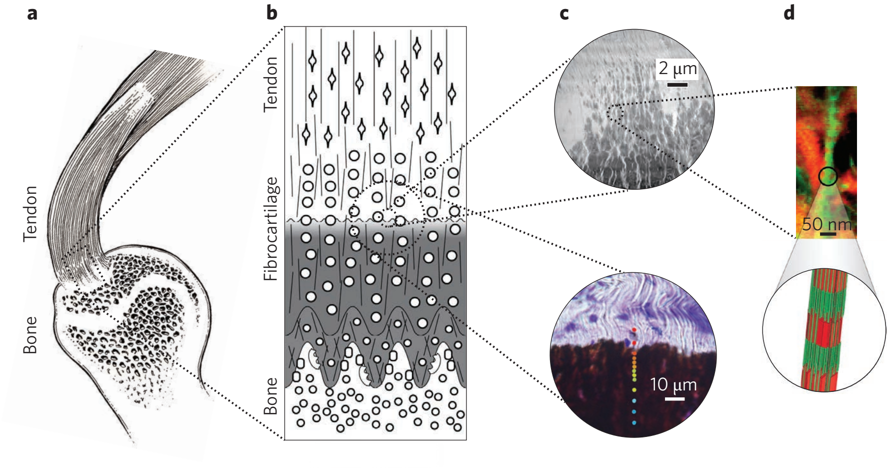

Figure 1 | The hierarchical structure of the tendon-to-bone attachment. **a**, Tissue-level schematic of the tendon-to-bone attachment. **b**, Schematic of transitional tissue, with mineral content within fibrocartilage indicated by greyscale intensity. **c**, TEM image of mineral gradient (top) and Raman microprobe results of mineral content (bottom), with colour dots indicating mineral content (red, low; blue, high). **d**, TEM-electron energy loss spectroscopy image revealing calcium-dominated regions (mineral) in red and carbon-dominated regions (tropocollagen) in green. Tendon inserts into bone over what can appear as a disordered, smooth or sharp fibrocartilaginous transition, depending on the length scale of observation. At both the highest and lowest length scales (**a** and **d**, respectively), the tissue and transitions appear ordered and smooth. However, at the intermediate mesoscale (~100 nm to ~10 µm; **c**), reports of important roles of stochastic material distributions have emerged. The results of Rossetti *et al.*[11] solidify the picture of randomness dominating over order in the mechanisms of strength and resilience at the tendon-to-bone attachment by revealing the scaffold on which cells and mineral interact to behave like a random fibrous network. Adapted from ref 20, ASME (**b**); ref. 12, PLoS (**c**, top); ref. 13, Elsevier (**c**, bottom); ref. 3, Royal Society (**d**).

Joint motion is achieved through the transfer of muscle load from tendon to bone — two tissues with dramatically different compositions, structures and mechanical properties. From the continuum perspective, the Poisson ratios for stretching in this direction can differ by nearly one order of magnitude, while the moduli of tendon and bone along the direction of muscle force differ by nearly a hundredfold[1]. From the structural perspective, both are hierarchically organized, with nanoscale tropocollagen triple helices bundling into micrometre-diameter fibrils, which in turn bundle into fibres hundreds of micrometres in diameter and many millimetres long. In bone, the fibrils are stiffened by the cross-linking caused by the insertion and coating of bioapatite mineral crystals[2,3]. The tendon-to-bone attachment would be expected to fail from Bogy- or Williams-type free-edge singularities if stress concentrations at the interface were not attenuated with a suite of cross-scale deformation mechanisms[4]. Indeed, the rates of failure after surgical repair pose a major challenge[5]. How, then, does the healthy tendon-to-bone attachment achieve effective load transfer from one tissue to the other, and why does the healing attachment fail so frequently? The answer lies in a unique hierarchical transitional tissue that exists at the interface between these dissimilar materials, and identifying and restoring the resilience mechanisms of this material is now a major target for tendon-to-bone repair strategies[6].

This transitional tissue was originally described as having four distinct zones (tendon, fibrocartilage, mineralized fibrocartilage, and bone)[7]. However, as refined characterization techniques became available, this view more recently gave way to a picture of a continuous, functionally graded tissue. Spectroscopic microprobe analyses showed a smooth transition in mineralization between tendon and bone[8,9]. Homogenization models showed that smooth gradients in composition and alignment of partially mineralized collagen fibres could account for transitional mechanical properties between tendon and bone that enabled reduction of stress concentrations[10].

The structural, compositional and mechanical observations that Rossetti *et al.* now report in *Nature Materials* complete this developing picture[11]. Randomness has now emerged as a key structural feature of effective stress transfer between the two tissues; identified previously in mineral deposits[12] and interdigitation[13], and now shown by Rossetti and colleagues in collagen organization. Transmission electron microscopy (TEM) imaging of the tendon-to-bone attachment at the nanoscale revealed small bioapatite pockets that gave the illusion of smooth grading[12] (Fig. 1c). Imaging at larger length scales revealed roughness, with stochastic interdigitation between tendon and bone[13] (Fig. 1b,c). Similarly, a role of collagen disorganization in attachment mechanics has long been known[10]. The final piece of the puzzle is provided by Rossetti and colleagues through a new picture of the fibrous nature of the enthesis. The authors report that the enthesis behaves not as an affinely deforming connective tissue, as tendon and earlier tendon-to-bone models have predicted[10], but instead as a networked solid, in which only a fraction of fibres form a contiguous network to resist loading in a particular

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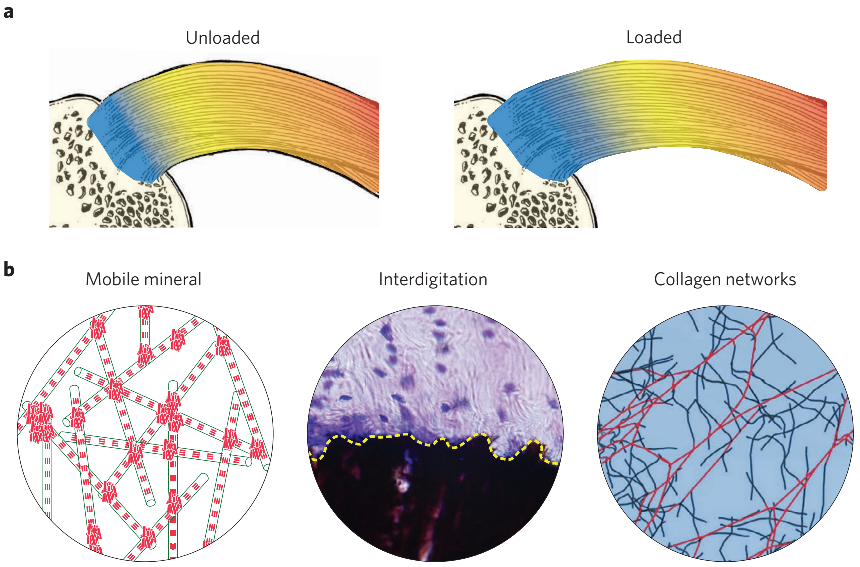

Figure 2 | The random, fibrous nature of the tendon-to-bone attachment endows it with toughness while protecting tendon and bone. **a**, The disordered, energy-absorptive barrier model. Deformation localizes to the enthesis site (blue) due to its high compliance relative to bone (tan), tendon (yellow) and muscle (red). This high compliance arises in part from the character of the tissue at the attachment site, which is now known to behave like a fibrous network. **b**, This tissue is expected to be exceptionally tough relative to the neighbouring tissue because of the three known components of disorder at the enthesis site: randomly distributed, mobile mineral (red plates); interfacial roughness (yellow dashed line); and the newly identified disordered fibre arrays (loaded fibres in red; unloaded fibres in blue). Panel **b**, right, adapted from ref. 17, Elsevier.

direction[14–17]. This was observed as the fibres within a tendon-to-bone insertion site accepted and shed load depending on the direction of loading. This new result shows that the enthesis deforms as a fibrous network rather than as an affine continuum, and the new picture (Fig. 1) has significant implications for the nature of dissimilar bimaterial attachments.

The long-suspected model of the enthesis serving as a compliant band with exceptional energy absorption and resilience to failure is enhanced by this new picture, with every length scale of the hierarchical enthesis tailored to ensure a contribution towards higher toughness (Fig. 2). At the micrometre scale, Rossetti *et al.* demonstrate that this networked solid becomes less stiff than the tissue surrounding it by offloading many of the fibres. This dramatically amplifies the effects of orientation identified previously, and might be a key factor in the development of a stress-alleviating compliant band between tendon and bone[18]. The fibrous nature of the tissue reduces stiffness beyond what would be expected by the homogenization models that have been applied previously, and probably accounts for the failure of these models to show the degree of stiffness disruption observed experimentally. At the nanoscale, the fibrous nature of the enthesis suggests an even greater role of mobile mineral than previously hypothesized[19]. Mineral sliding along fibres has been assumed to toughen partially mineralized tissues, and the finding of a networked solid suggests an even more important role, with mineral configurations probably mediating the ways that fibres are reoriented and recruited to transfer load. Furthermore, the recruitment and successive failure of distinct fibre networks would be expected to impart additional toughness on the attachment[14–17], comparable to the way microscale interdigitation of tendon and bone toughens the attachment, and analogous to the way that brittle matrix fibrous composites achieve their toughness.

The structural, compositional and mechanical features described here and by Rossetti *et al.* result in a transitional tissue between tendon and bone that is the toughest component of the tendon–enthesis–bone linkage, with the newly identified fibrous nature of the enthesis enabling every part of the tissue to absorb energy while resisting failure. The most compliant tissue of the attachment is thus the tissue in which mineral is below the percolation threshold: deformation at high loads becomes localized in the region with the highest mineral mobility and the most fibrous character, enabling multiple hierarchies of failure through the stochastic nature of load engagement and redistribution in a network of random fibres. The newly identified fibrous character of the attachment site adds a critical missing link in the story of how this tissue serves as a protective band that is simultaneously compliant and tough, and solidifies the new model of the tendon-to-bone attachment as a connection that unifies two highly organized, dissimilar materials through a disordered tissue system. ❐

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