IBMS BoneKEy. 2011 June;8(6):271-285
http://www.bonekey-ibms.org/cgi/content/full/ibmske;8/6/271
doi: 10.1138/20110515

PERSPECTIVES

The Role of Mechanobiology in the Attachment of Tendon to Bone

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Abstract

The attachment of dissimilar materials is a major engineering challenge. Stress concentrations would arise at the interface of a compliant tendon and a stiff bone unless the interface was tuned to this mechanical mismatch. The functionally graded tissue that develops postnatally between tendon and bone provides a robust attachment that alleviates potential stress concentrations. This unique transitional tissue is not recreated during healing, so surgical reattachment of these two tissues often fails. “Mechanobiology”, the response of cells to changes in their mechanical environment, plays an important role in the development and homeostasis of musculoskeletal tissues. This *Perspective* reviews work examining structure-function relationships at the tendon-to-bone insertion and the role of mechanobiology in the development and healing of the tendon-to-bone insertion site. Results indicate that the tendon-to-bone insertion is a functionally graded material with regard to its extracellular matrix composition, its structural organization, its mineral content, and its mechanical properties. Animal models examining the development of the insertion indicate that mechanical loading is necessary for the maturation of the insertion into a functionally graded material. The role of loading tendon-to-bone during healing is more complex; low levels of load are beneficial and high levels of load are detrimental to healing. A better understanding of mechanobiology at the insertion may lead to rehabilitation and tissue engineering strategies for enhancing tendon-to-bone healing. *IBMS BoneKEy*. 2011 June;8(6):271-285.

Keywords: Tendon-to-bone; Mechanobiology; Enthesis; Functionally graded material

Introduction

Tendons insert into bone across either fibrous or fibrocartilaginous transitions (the “enthesis”) (1). Fibrous transitions are characterized by broad insertions (effectively distributing the forces over large areas and reducing stresses) and by perforating mineral fibers (“Sharpey’s fibers”) (2). Examples of these attachments include the tibial insertion of the medial collateral ligament and the deltoid tendon insertion into the humeral head. Examples of the more common fibrocartilaginous insertions include the rotator cuff tendon insertions and the Achilles tendon insertion. The structure, function, development, and healing of fibrocartilaginous tendon-to-bone insertions will be the focus of this *Perspective*. These insertions are characteristically short and occur across distinct fibrocartilage regions with gradations in collagen structural organization, extracellular matrix composition, mineral content, and mechanical behavior (Fig. 1) (1). A high incidence of recurrent failure has been reported following surgical repair of tendon to bone (3;4). The high failure rates are likely due to an inability to reconstruct the complexity of the uninjured enthesis during healing (5-7). Mechanobiology plays a critical role in the development, maintenance, and healing of musculoskeletal tissues. This has been demonstrated most clearly in bone, but is also apparent in tendon, cartilage, and muscle. Mechanobiology plays a role at all of the tendon-to-bone insertion site hierarchical levels: at the organ level, the architecture of the insertion has been shown to provide stress transfer that reduces the stress concentrations that normally arise at

bi-material interfaces (8;9); at the tissue level, a continuous gradation is seen in structure, composition, and mechanical properties (8;10); at the fiber level, gradations lead to well-defined mechanical functions (11); and at the micro-scale, mechanical factors have been shown to control cell behavior (12). Animal models have demonstrated that mechanobiologic factors are central to the formation of a gradation in properties from tendon to bone during development and critically affect healing (7;13-15). This *Perspective* presents work that examines the role of the mechanical environment in the development and healing of the tendon-to-bone insertion site.

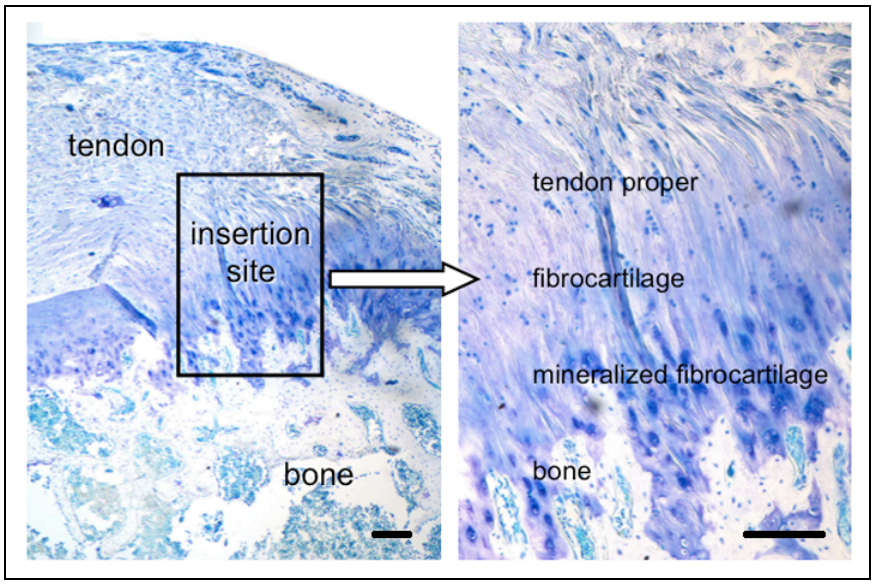

Fig. 1. Tendon attaches to bone across a functionally graded fibrocartilaginous transition site (toluidine blue-stained section from a rat supraspinatus tendon-to-bone insertion is shown; scale bar = 200mm). (Figure modified, with permission, from: Thomopoulos S, Hattersley G, Rosen V, Mertens M, Galatz L, Williams GR, Soslowsky LJ. The localized expression of extracellular matrix components in healing tendon insertion sites: an *in situ* hybridization study. *J Orthop Res*. 2002 May;20(3):454-63).

Structure-Function Relationships

The mechanical challenge of attaching tendon to bone

The mechanical properties of tendon are vastly different from those of bone (16;17). The tensile modulus of tendon is ~200MPa in the direction of muscle force, but buckles in compression (*i.e.*, it behaves like a “rope”) (17). In contrast, bone has a modulus of 20GPa in both tension and compression (16). Stress concentrations can be expected to arise at the attachment point of these two disparate materials unless the insertion is optimized to overcome the stiffness mismatch. The tendon-to-bone insertion site overcomes this challenge via gradations in its composition and structure, leading to a functional gradation in mechanical properties. In a series of studies examining the rat supraspinatus tendon-to-bone insertion, gradations were demonstrated in extracellular matrix gene expression, collagen fiber orientation, mineral content, and mechanical properties (8;10;11;18). Localized gene expression was determined using *in situ* hybridization (10). The expression of fibrillar collagens varied along the insertion: tendon fibroblasts expressed type I collagen, fibrochondrocytes in the transitional tissue between tendon and bone expressed type II and X collagen, and osteoblasts in bone expressed type I collagen. Consistent with a fibrocartilaginous morphology, aggrecan expression was also localized to the insertion site. Using a quantitative polarized microscopy technique, collagen fibers were found to be less oriented at the insertion compared to the

tendon (10). In a separate study, mineral content along the insertion was determined using Raman microprobe analyses (18). Based on the intensity of the phosphate peak relative to a collagen-specific organic peak, it was determined that there was an approximately linear increase in mineral content across the interface, from tendon to bone. Biomechanical tests demonstrated that the tensile stiffness (*i.e.*, the slope of the linear portion of the stress-strain plot) of the insertion was approximately half that of tendon. Stiffness therefore decreases significantly as the tendon inserts into bone before increasing to the level of bone. This compliant region may serve to dissipate stress concentrations that would otherwise arise at the interface between the two mechanically dissimilar materials. These results in the rat supraspinatus tendon enthesis are consistent with reports examining other soft tissue-to-bone attachments (19-21). At the anterior cruciate ligament-to-bone insertion, microcompression was performed to determine the mechanical properties and energy dispersive x-ray analysis was performed to determine mineral content (20). The calcified regions of the insertion exhibited significantly greater compressive moduli than the noncalcified regions. Similar results were reported for meniscal attachments to bone (22;23). Based on these studies, it is apparent that the tendon-to-bone insertion site varies dramatically along its length in collagen structural arrangement, extracellular matrix composition, mineral content, and mechanical properties. The complex composition, structure, and mechanical behavior of the tendon-to-bone insertion results in a particularly difficult challenge for effective response to injury**.**

Mechanisms for overcoming the mechanical mismatch between tendon and bone

Four distinct strategies have been identified for achieving effective load transfer between tendon and bone: 1) a shallow attachment angle at the insertion of tendon into bone; 2) shaping of tissue morphology of the transitional tissue; 3) interdigitation of transitional tissue with bone; and 4) functional grading of transitional tissue between tendon and bone (8;9;11). The degree of the stress singularity at an interface between tendon and bone depends on the angle of attachment; a shallow angle of attachment ameliorates the stress singularity. Peak stresses are also reduced via optimization of the gross shape of the insertion. Interlocking of the tissues through interdigitation (24) increases the toughness of the interface. The variation in structure and composition along the insertion results in a unique grading of mechanical properties. A mechanical model of the insertion demonstrated that two of these features, mineral content and collagen organization, combine to produce a functionally graded insertion (11). The linear increase in mineral accumulation on collagen fibers provides significant stiffening of the fibers, but only for concentrations of mineral above a “percolation threshold” corresponding to formation of a mechanically continuous mineral network. The decrease in collagen fiber orientation is a second major determinant of tissue stiffness. The combination of these two factors results in the previously reported variation of stiffness over the length of the tendon-to-bone insertion. The four mechanisms described above provide a nano- through macro-mechanical understanding of how a robust tendon-to-bone attachment is achieved. This understanding may guide surgical and tissue engineering strategies for improving tendon-to-bone healing.

The Role of Mechanobiology During Development

The role of mechanobiology in musculoskeletal tissue development and homeostasis

Biophysical cues influence fetal joint development (25-28). Abnormal loading during fetal or early postnatal timepoints can result in a number of pathologies. For example, traction injury to the brachial plexus during the birth process results in a muscle imbalance across the developing shoulder and leads to a number of complications (29). The response of adult

musculoskeletal tissues to mechanical loading is also well-described (30;31). Experimental and modeling studies demonstrated that the architecture of trabecular bone and the thickness of cortical bone were highly dependent on the stress environment (31;32). A similar responsiveness to the mechanical environment was demonstrated in tendon. Removal of load resulted in rapid deterioration of tendon strength and a change in structure and composition (33-36). A change in the stress *mode* has also been shown to affect tendon properties. Areas of tendon that were subjected to compression (*e.g.*, the portion of the rotator cuff directly under the acromion and locations where flexor tendons wrap around bony pulleys) produced significant amounts of proteoglycans to resist deformation in the direction of compression (37;38). As all of the cell types found along the tendon-to-bone insertion are sensitive to their mechanical environment, a role for mechanobiology is expected at the developing enthesis.

The role of mechanobiology in tendon-to-bone insertion development

The enthesis forms through endochondral ossification, with expression of Indian hedgehog, parathyroid hormone-related peptide receptor, and collagen X seen at various stages of development (39-45). Blitz *et al*. demonstrated that this process occurs through two phases: initiation is regulated by biologic signals from the tendon and growth is regulated by mechanical signals from the muscle (40). The transcription factor scleraxis regulates the growth factor bone morphogenetic protein 4 to initiate the formation of the enthesis at the deltoid tuberosity in a muscle-independent manner. Muscle loads are only necessary for the subsequent growth and maturation stages of enthesis development. *In situ* hybridization studies have also revealed the spatial and temporal expression patterns of extracellular matrix genes during rotator cuff tendon enthesis development. Expression patterns for collagen I (characteristic of fibroblasts and osteoblasts), collagen II (characteristic of chondrocytes), and collagen X (characteristic of hypertrophic chondrocytes) have been studied (45). Neotendon was evident adjacent to bone at 15.5 days post-conception. A transition zone between the tendon and bone began to form 7 days postnatally and reached a mature morphology by 21 days postnatally. The fibroblasts of the tendon expressed type I collagen at all timepoints. The chondrocytes in the unmineralized bone expressed type II collagen until 14 days postnatally. The chondrocytes near the insertion became hypertrophic at this point and began expressing type X collagen. Type I collagen and type II collagen were expressed by two adjacent populations of cells: collagen I was expressed by the fibroblasts on the tendon end of the insertion, while collagen II was expressed by the chondrocytes on the bony end of the insertion. Type X collagen expression was not turned on until 14 days postnatally. Notably, many of the critical events in rotator cuff tendon-to-bone insertion development occurred at postnatal timepoints. Similar results have been reported for the anterior cruciate ligament enthesis (39) and for the Achilles tendon enthesis (43;44).

To examine the role of mechanical loading on the postnatal development of the tendon-to-bone insertion, rotator cuff muscles were paralyzed using either intramuscular injections of botulinum toxin A (“BTX” group) or laceration of the upper trunk of the brachial plexus (“neurotomy group”) (13;46-48). The neurotomy group was used to determine if there were any direct effects of botulinum toxin on rotator cuff development (*i.e.*, other than the effects of muscle unloading). Paralysis was induced within 24 hours of birth. In the botulinum toxin-injected mice, the muscles of contralateral shoulders were injected with saline to serve as controls (“saline” group). Local paralysis in these mice was maintained until sacrifice through repeated botulinum toxin injections. A separate group of neonatal mice was injected with saline in both shoulders and served as fully mobile controls (“normal” group).

Geometric and functional assessment of muscles demonstrated that both methods of

muscle paralysis led to dramatic decreases in loading across the developing supraspinatus tendon-to-bone insertion (48). The mass and volume of supraspinatus muscles injected with botulinum toxin was decreased by more than 66% compared to the contralateral saline-injected muscles at 56 days. Similar changes in muscle volume were seen in the neurotomy group (47). Decreases in muscle mass and volume corresponded to decreases in muscle force generation. After 28 and 56 days of postnatal paralysis, saline-injected and normal supraspinatus muscles generated dramatically higher forces compared to botulinum-injected muscles.

Reduced muscle loading impairs mineral deposition, fibrocartilage formation, and leads to disorganized fiber distribution and inferior mechanical properties at the tendon enthesis

The accumulation of mineral in the humeral head during postnatal development was decreased when loading was removed (Fig. 2) (13;47).

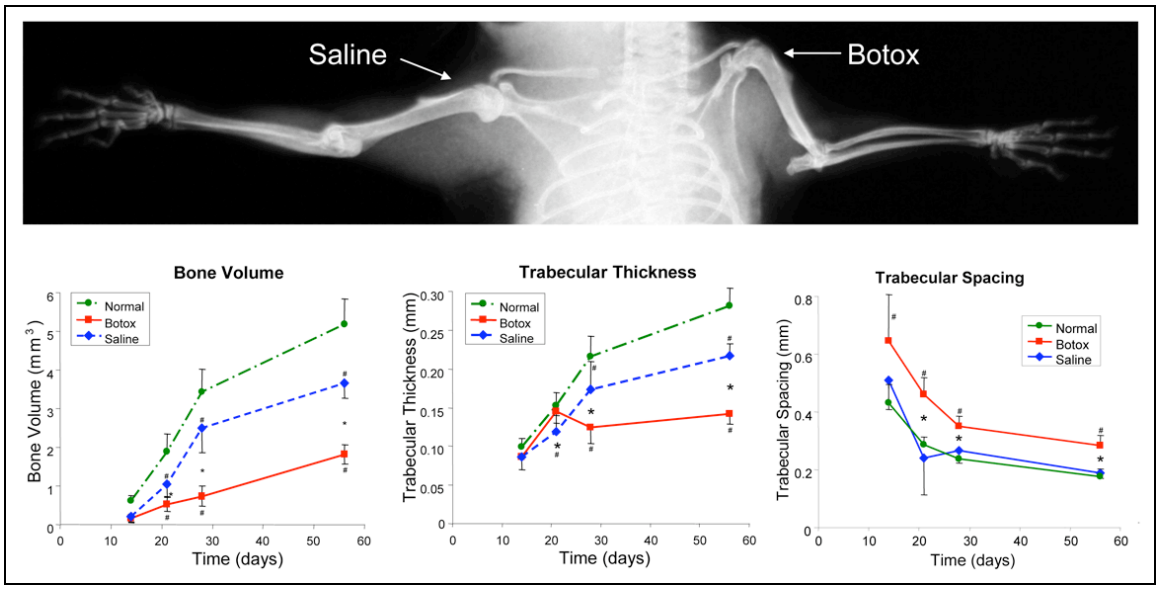

Fig. 2. Bony deformities were evident radiographically in the humeral head of botulinum toxin-injected shoulders after 56 days of postnatal development. Bone volume and trabecular architecture were significantly different in the botulinum toxin-injected group compared to the saline-injected and normal groups (*p < 0.05 saline-injected vs. botulinum toxin-injected, #p < 0.05 saline-injected or botulinum toxin-injected vs. normal). (Figure modified, with permission, from: Thomopoulos S, Kim HM, Rothermich SY, Biederstadt C, Das R, Galatz LM. Decreased muscle loading delays maturation of the tendon enthesis during postnatal development. *J Orthop Res*. 2007 Sep;25(9):1154-63).

Micro-computed tomography analysis revealed dramatic differences in the amount of mineralized bone when comparing saline-injected to botulinum toxin-injected shoulders, demonstrating the sensitivity of bone to its mechanical environment. Similar results were found after neurotomy. Interestingly, no differences were seen in any bone measure when comparing saline and BTX at 14 days. However, differences between the two groups were significant for most measures at the later timepoints. The lack of mineral accrual in unloaded insertions was at least in part due to increased levels of bone resorption, as evidenced by increased osteoclast numbers in the BTX group compared to the saline group. To further test the role of osteoclast activity in this process, osteoclast activity was suppressed in a group of animals using alendronate (a bisphosphonate that blocks osteoclast activity). Increasing levels of alendronate led to higher bone volume and higher connectivity density in a dose-dependent manner in the BTX group compared to the saline group, partially

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rescuing the defects caused by unloading (Fig. 3). These results demonstrate the sensitivity of mineralization to mechanical loading in the developing tendon-to-bone insertion.

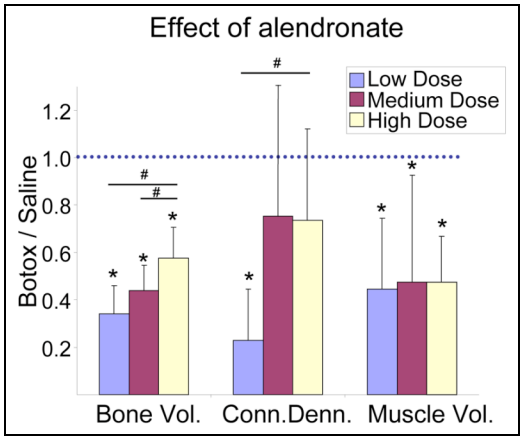

Fig. 3. Increasing levels of alendronate (a bisphosphonate that suppresses osteoclast activity) led to higher bone volume and higher connectivity density in a dose-dependent manner, partially rescuing the detrimental effects of botulinum toxin-induced unloading. There was no apparent effect of alendronate on muscle volume. (*p < 0.05 botulinum toxin-injected vs. saline-injected, #p < 0.05 for dose effect; doses: 0.125, 1, and 2 mg/kg/week).

A zone of hypertrophic chondrocytes was evident between the supraspinatus tendon and its humeral head insertion until 14 days postnatally, regardless of loading conditions (13). By 21 days, however, there were clear differences in fibrocartilage maturation when comparing the BTX group to the saline group (Fig. 4).

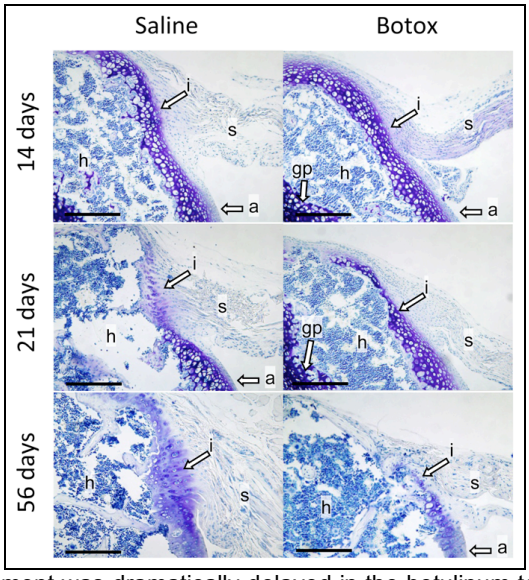

Fig. 4. Fibrocartilage development was dramatically delayed in the botulinum toxin-injected group compared to the saline-injected group. Hypertrophic chondrocytes were seen at the insertion (indicated by the arrow) at 14 days in both groups and at 21 days in the Botox group (i, tendon-to-bone insertion; s, supraspinatus tendon; a, articular surface of the humeral head; h, humeral head; gp, growth plate; scale bar = 200mm). Sections are stained with Toluidine blue; cell nuclei are stained blue and fibrocartilage matrix is stained purple. (Figure modified, with permission, from: Thomopoulos S, Kim HM, Rothermich SY, Biederstadt C, Das R, Galatz LM. Decreased muscle loading delays maturation of the tendon enthesis during postnatal development. *J Orthop Res*. 2007 Sep;25(9):1154-63).

The fibrochondrocytes at the insertion were arranged in a columnar pattern perpendicular to the subchondral bone plate. Contralateral botulinum toxin-injected shoulders showed no fibrocartilage between the tendon and bone. A layer of disorganized mesenchymal-like cells and hypertrophic chondrocytes was present at the insertion site. The tendon enthesis was fully developed in the saline group by 56 days. In contrast, the tendon enthesis in the BTX group remained disorganized with a sparse amount of fibrocartilage. Consistent with the previous study examining mineral accumulation, removal of mechanical loading cues postnatally dramatically impaired the fibrocartilage formation at the tendon-to-bone insertion.

Collagen fibers in both the tendon and the insertion were less organized in the BTX group compared to the saline group at 28 and 56 days. Tensile mechanical loading therefore plays a critical role in directing collagen alignment at the insertion. Biomechanical testing of the tendon-to-bone insertion demonstrated that the mechanical properties of the BTX group were significantly inferior to those of the saline group at 56 days. In summary, the formation of a functionally graded transition between tendon and bone requires the presence of physiologic muscle loading.

The Uncertain Role of Mechanobiology During Healing

The influence of the loading environment on tendon-to-bone healing is unclear

Tendon-to-bone healing is characterized by the formation of connective tissue with vastly inferior mechanical properties compared to the normal, uninjured tendon enthesis (7). Rotator cuff injuries, which make up the majority of soft tissue injuries to the upper extremity, commonly require repair of one or more tendons to humeral head bone (49). Anterior cruciate ligament reconstruction techniques typically use tendon grafts that must heal in tibial and femoral bone tunnels (50). In most cases of tendon-to-bone healing, clinical outcomes have been disappointing. At the rotator cuff, for example, the recurrence of tears to repaired tendons has been reported to range from 24-94% (3;4). Studies in the rabbit (51), the goat (52), and the rat (5;7) have demonstrated that the mechanical properties of the healed tissue are dramatically weaker than normal. While the poor healing is likely due to a number of factors, including bone loss (53;54) and lack of collagen integration into bone, the most dramatic observation in these animal models was the lack of a transitional zone between the tendon and bone (Fig. 5) (5-7).

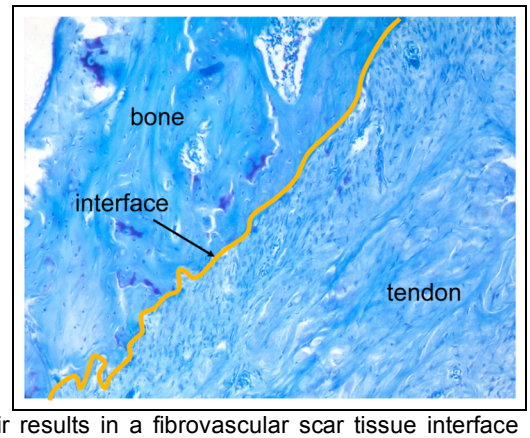

Fig 5. Tendon-to-bone repair results in a fibrovascular scar tissue interface rather than regeneration of a functionally graded insertion (toluidine blue-stained section is shown for a repaired supraspinatus tendon-to-bone insertion after 28 days of healing). (Figure modified, with permission, from: Thomopoulos S, Hattersley G, Rosen V, Mertens M, Galatz L, Williams GR, Soslowsky LJ. The localized expression of extracellular matrix components in healing tendon insertion sites: an *in situ* hybridization study. *J Orthop Res*. 2002 May;20(3):454-63).

The role of mechanobiology during the healing process is unclear. Increased static stress was shown to be beneficial to the properties of the healing medial collateral ligament (MCL) in one animal study (55). Increased cyclic stress, on the other hand, was found to be detrimental to healing in a rabbit MCL mid-substance model (56). Increased passive motion applied to flexor tendon mid-substance injuries, on the other hand, has been shown to be beneficial to healing (7;57). While all three components of the insertion site (tendon, fibrocartilage, and bone) are responsive to load when healthy, their response to load during tendon-to-bone healing has required further study.

Cast immobilization is beneficial to tendon-to-bone healing compared to exercise

In order to examine the role of mechanical loading on tendon-to-bone healing, supraspinatus tendons of rats were detached and repaired to their bony insertions (7;14;58;59). Post-operative activity level was controlled in three groups: cast immobilization, unrestrained cage activity, and treadmill exercise. A separate group of uninjured animals served as normal, un-operated controls. The healing interface consisted of a fibrovascular scar tissue in all groups. Recreation of a functionally graded transition was not seen in any case. Geometrically, the cross-sectional area was significantly increased in all injury groups compared to the uninjured control. As expected, increased activity stimulated matrix formation, leading to larger cross-sectional area in the exercised group compared to the cast-immobilized group. Biomechanically, there were significant changes in mechanical properties due to injury and due to activity level. The mechanical properties in all injury groups were dramatically lower than those of the uninjured group. Surprisingly, increased activity led to lower mechanical properties. Collagen fiber organization approached normal by 16 weeks of healing in the immobilized group, while the exercised group remained poorly organized. When examining composition, the expression of extracellular matrix genes was closest to normal in the cast-immobilized group. Importantly, increases in joint stiffness due to immobilization were transient; shoulder range of motion returned to normal in the long-term (58). Attempts to modulate the post-operative loading regimen in this animal model did not result in any improvements in healing. Immediate loading after repair via passive motion (rather than exercise) resulted in increases in joint stiffness (59). Using a rat model of anterior cruciate ligament reconstruction, studies on tendon-to-bone healing implicated cytokines produced by infiltrating macrophages as likely contributors to poor healing outcomes (60). Macrophage depletion following anterior cruciate ligament reconstruction resulted in improved healing at the tendon-to-bone interface (61). Recent work has demonstrated that improved healing via immobilization is in large part due to a suppression of macrophage accumulation, leading to improved tendon-bone integration (62).

These data suggest that cast immobilization is beneficial to tendon-to-bone healing compared to exercise. Increased loading may be effective in stimulating matrix synthesis, but it is ineffective in improving the mechanical properties of the repair. More material of lesser quality was produced when loading was increased. The insertion site failed to heal properly, with long-term biomechanical, structural, and compositional properties significantly different from uninjured controls. A key feature of the failed healing response was the lack of a functionally graded transition between the tendon and the bone (Fig. 5). Instead, the transition zone was composed of disorganized scar tissue. These studies demonstrated that the tendon-to-bone insertion site heals poorly but is sensitive to its mechanical environment.

Complete removal of load is detrimental to tendon-to-bone healing

The positive outcomes in the cast-immobilized group led to further investigations of the role of mechanical loading in tendon-to-bone healing. Cast immobilization, while significantly reducing

the load across the healing repair site, does not result in a zero loading state. The supraspinatus muscle was fully innervated, and therefore able to generate load across the repair site in the absence of shoulder motion. The effect of *complete* removal of load on tendon-to-bone healing was examined using two animal models (63-65). It was hypothesized that complete unloading would be detrimental to healing due to the lack of mechanical signals to guide matrix synthesis and collagen fiber alignment. In the first animal model, the supraspinatus tendons of rats were injured and repaired (63;65). The healing supraspinatus muscle was then paralyzed with botulinum toxin and the shoulder was cast-immobilized post-operatively. The combination of muscle paralysis with cast immobilization was detrimental to healing, as evidenced by decreased mechanical properties (Fig. 6). The structural properties of the healed tendons were significantly greater in the saline-injected specimens compared to the botulinum toxin-injected specimens. This study showed that when *all* load is removed from the healing tendon (by paralyzing the supraspinatus muscle and casting the shoulder) the cross-sectional area and the structural properties are decreased compared to protective cast-immobilization alone.

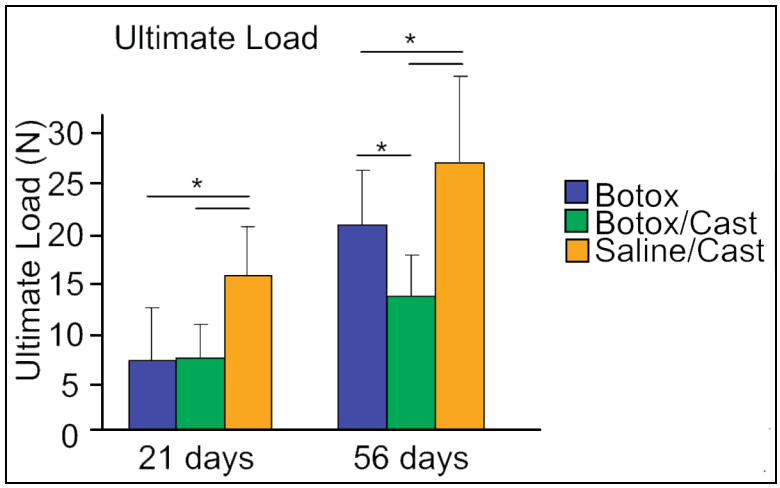

Fig. 6. Ultimate load was higher in the saline-injected/casted group at 21 and 56 days compared with the botulinum toxin-injected and botulinum toxin-injected/casted groups. Load in the botulinum toxin-injected group was significantly higher than the botulinum toxin-injected/casted group at 56 days (*$p < 0.05$). (Figure modified, with permission, from: Galatz LM, Charlton N, Das R, Kim HM, Havlioglu N, Thomopoulos S. Complete removal of load is detrimental to rotator cuff healing. *J Shoulder Elbow Surg*. 2009 Sep-Oct;18(5):669-75).

The effect of complete removal of load on tendon-to-bone healing was also examined using a canine flexor tendon injury and repair model (64). Repaired tendons were either cut proximally to remove load from the distal phalanx repair site or left intact proximally. All paws were cast-immobilized post-operatively. Specimens were tested to determine range of motion, mechanical properties, repair-site gapping, and bone mineral density. As with the rat rotator cuff study, complete removal of load across the repair site was detrimental to healing, as evidenced by decreased range of motion and decreased mechanical properties. Loading did not affect bone mineral density or gapping. Although reduced loading (*e.g.*, through cast immobilization) can be beneficial to healing (presumably by eliminating excessive motion at the repair site), some load applied to the site via normal muscle contraction is necessary for effective healing.

Controlled periods of immobilization and re-mobilization after tendon-to-bone repair

To further elucidate the relationships between mechanical loading and tendon-to-bone healing, Stasiak *et al*. developed a

knee joint fixation/distraction system to apply controlled loads to *in vivo* rat anterior cruciate ligament reconstructions (66). The system was used to examine the effect of controlled axial loading on tendon-to-bone healing (67;68). Controlled cyclic axial loading was applied after a period of immobilization; this rehabilitation protocol led to improved tendon-to-bone healing compared to immediate axial loading or prolonged immobilization. Improved properties were likely due to significantly fewer inflammatory macrophages and significantly more resident macrophages at the healing tendon-bone interface in the delayed-loading group compared with the immediate-loading and immobilization groups. In contrast to these results, exercise following a short immobilization period led to increases in joint stiffness and decreases in repair-site mechanical properties in a rat rotator cuff model (69). These studies demonstrate the difficulty in modulating tendon-to-bone healing through mechanobiology.

Conclusion

The attachment of tendon to bone presents a major mechanical challenge due to the two orders-of-magnitude difference in the mechanical properties of the two tissues. The natural tendon enthesis overcomes this mechanical mismatch by attaching the two tissues across a functionally graded transitional tissue. This unique transition develops between tendon and bone post-natally, and is driven in large part by mechanical factors. In the absence of muscle loading during development, mineral deposition is decreased, fibrocartilage does not form, collagen fiber deposition is disorganized, and mechanical properties are decreased. Tendon-to-bone healing is characterized by the formation of a fibrovascular scar without the regeneration of a functionally graded insertion. This results in repaired attachments that are prone to rupture. The healing insertion is also sensitive to its mechanical environment. Studies in animal models, however, have demonstrated that a fine balance must be reached between too much load (which can lead to microdamage) and too little load (which can lead to a catabolic environment) to maximize tendon-to-bone healing. Ultimately, approaches for improving tendon-to-bone healing must focus on recreation of a functionally graded transition between tendon and bone, either through manipulation of the loading environment or though an engineered construct.

Acknowledgment

Grants from the National Institutes of Health (AR055580), the National Science Foundation (CAREER 844607), and the Orthopaedic Research and Education Foundation supported this work.

Conflict of Interest: None reported.

Peer Review: This article has been peer-reviewed.

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