Perspectives

Mechanisms of tendon injury and repair[†]

Stavros Thomopoulos[1*]
William C. Parks[2]
Daniel B. Rifkin[3]
Kathleen A. Derwin[4*]

[1]Washington University, Department of Orthopedic Surgery, Saint Louis, MO
[2]Cedars Sinai Medical Center, Department of Medicine, Los Angeles, CA
[3]New York University, Department of Cell Biology, New York, NY
[4]Cleveland Clinic Lerner Research Institute, Cleveland, OH

***** Corresponding Authors:
Stavros Thomopoulos, Ph.D.
Washington University
Department of Orthopaedic Surgery
660 South Euclid, Campus Box 8233
St. Louis, MO 63110
Phone: 314-362-8605
Email: ThomopoulosS@wustl.edu

Kathleen A. Derwin, Ph.D.
Cleveland Clinic Lerner Research Institute
Department of Biomedical Engineering
9500 Euclid Avenue
Cleveland, Ohio 44195
Phone: 216-445-5982
Email: DerwinK@ccf.org

Keywords: animal models, inflammation, healing, tendinopathy

Received 22 November 2014; Revised; Accepted 13 December 2014
Journal of Orthopaedic Research

DOI 10.1002/jor.22806

Abstract

Tendon disorders are common and lead to significant disability, pain, healthcare cost, and lost productivity. A wide range of injury mechanisms exist leading to tendinopathy or tendon rupture. Tears can occur in healthy tendons that are acutely overloaded (e.g., during a high speed or high impact event) or lacerated (e.g., a knife injury). Tendinitis or tendinosis can occur in tendons exposed to overuse conditions (e.g., an elite swimmer's training regimen) or intrinsic tissue degeneration (e.g., age-related degeneration). The healing potential of a torn or pathologic tendon varies depending on anatomic location (e.g., Achilles vs. rotator cuff) and local environment (e.g., intrasynovial vs. extrasynovial). Although healing occurs to varying degrees, in general healing of repaired tendons follows the typical wound healing course, including an early inflammatory phase, followed by proliferative and remodeling phases. Numerous treatment approaches have been attempted to improve tendon healing, including growth factor- and cell-based therapies and rehabilitation protocols. This review will describe the current state of knowledge of injury and repair of the three most common tendinopathies-- flexor tendon lacerations, Achilles tendon rupture, and rotator cuff disorders-- with a particular focus on the use of animal models for understanding tendon healing. . This article is protected by copyright. All rights reserved

Epidemiologyand Etiology of Tendon Injury

Flexor tendon injury occurs most commonly by laceration, with the highest incidence in persons aged 20-29 years, with a higher incidence in males than females.[1] Work-related injuries account for ~25% of acute traumatic flexor tendon injuries, most commonly in construction and extraction (44%), food preparation and serving (14%), and transportation and material moving (12%) occupations.[1] The Achilles tendon, the largest and strongest tendon in the human body, is involved in as much as half of all sports-related injuries. The vast majority (~75%) of Achilles tendon ruptures occur in men aged 30-49, and participating in a sports activity is the most common etiologic factor for injury.[2,3] Biopsies retrieved at surgery have demonstrated degenerative changes in most ruptured Achilles tendons[4], suggesting that Achilles tendon ruptures could be characterized as acute trauma of chronically degenerated tendons. Rotator cuff disorders are the most common causes of shoulder disability and are very common in the aging population[5]. Full-thickness rotator cuff tears are present in approximately 13% of individuals in their 50s[6], 25% of individuals in their 60s and 50% of individuals in their 80s[5]. The etiology of rotator cuff tearing is multifactorial and likely a combination of age-related degenerative changes[7] and micro/macrotrauma. Besides age, smoking, hypercholesterolemia, and family history have been shown to predispose individuals to rotator cuff tearing[5]. It should be appreciated that injuries to the flexor and rotator cuff tendons are intra-synovial and do not undergo spontaneous healing, whereas injury to the Achilles tendon is extra-synovial where fibrous tissue formation can and does occur after injury. Since the local environment and mechanisms of tendon injury are quite different among these three tendinopathic conditions, research questions and models must be framed in the context of these distinctions to produce clinically relevant studies that can eventually be translated to clinical care.

Animal Models of Tendon Injury and Repair

Animal models are the primary means by which fundamental and translational questions related to the complex processes of tendon injury, healing, and repair are investigated. In general, the specific research question should drive the choice of animal model (Table 1). Below, we provide some considerations for choosing appropriate animal models in tendon injury and repair research. The citations provided are intended to be representative of the various animal models and are by no means exhaustive.

Animal models for examining basic mechanisms of chronic tendon injury

Understanding the basic mechanisms of chronic tendon degeneration and subsequent injury would allow for the prevention and/or early treatment of ruptures. This is particularly relevant to the rotator cuff or Achilles tendon, as they typically advance through chronic, degenerative conditions over extended time prior to injury. Chronic tendon injuries are a common musculoskeletal problem in horses[8], however, naturally occurring equine flexor tendon injury is impractical for broad use as a research model because the severity of equine disease is highly variable and there are practical issues related to animal size, housing and cost. Hence, investigators have used various methods to artificially induce chronic tendon injuries in animal models. For overuse injuries, uphill or downhill treadmill running in rats or mice has been used to induce injury to the rotator cuff[9] or Achilles tendons[10], respectively. Other investigators have induced overuse injury by applying controlled fatigue loading directly to the patellar tendons of anesthesized rats[11] and mice[12]. Still others have used full-thickness, partial-width, laceration of the infraspinatus tendon in sheep to induce overstressed and stress-deprived portions of the

tendon[13]. Finally, collagenase injection has been used to induce chronic tendon injury in the rat[14] rabbit[15] and sheep[16] models. While all of these models capture important aspects of tendon degeneration and injury, it is important to keep in mind that none captures the complete etiology of the chronic tendon injuries seen in human patients. Therefore, care must be taken in the choice and interpretation of the animal model used.

Animal models for examining basic mechanisms of tendon healing

Understanding the basic mechanisms of tendon healing would inform the development of new treatments strategies for tendon repair. Animal models to investigateintra-tendinous healing in the absence of repair have largely been performed in rat and mouse models of Achilles, patellar, and flexor tendon injury, where injuries have been induced by a variety of methods, including full-width sharp[17] or blunt transection[18], punch biopsy or window defect[19], collagenase injection[14], partial-width incision[19], or needle stick[20]. The healing of intra-tendinous injury with repair has been investigated using mouse and rat modelsof flexor or Achilles tendon mid-substance tenotomy[21]or tenectomy[22]. The healing of tendon-to-bone repairhaslargely been studied in mouse and rat models of rotator cuff[23], Achilles[24] and flexor tendon[25] injury by sharp transection and then suture repair to bone. Tendon-to-bone healing has also been studied in the rabbit rotator cuff model[26]. The mouse is a particularly attractive animal model to study tendon healing due to the availability of a wide range of genetically manipulated targets thought to be involved in tendon healing and regeneration. Critical pathways of healing can therefore be studied in a mechanistic manner. A recent paper also describes tendon development in the zebrafish[27], introducing an additional animal model for studying tendon biology to the community that is even easier to manipulate genetically than the mouse. Although it is implicitly

assumed that the mechanisms observed in animal models are generalizable to the biology of human tendon healing, the extent to which the mechanisms of inducing injury, the particular tendon that is injured, or the age or species of the animal influence findings is currently unknown. Hence further work is needed to validate the generalizability and translatability of basic science studies of tendon healing in these animal models.

Animal models for translation to clinical care

A number of patient, surgical and post-operative influences should be considered in the choice and development of translational animal models for tendon repair and healing. The research variables of interest may include surgical technique, co-morbidities (e.g., obesity, smoking), repair augmentation strategies (e.g., grafts, cells, growth factors), and post-operative loading (rehabilitation). Because the goal of these research questions is translation to patient care, the animal model used should reflect the specific tendon of interest and incorporate clinically relevant features to the extent possible.Although the use of translational animal models may not allow for unraveling mechanistic links between functional outcomes and underlying biological events, results are intended to inform and improve clinical practice.

Flexor Tendon Repair

Translational studies of flexor tendon repair have largely been performed in the canine model. Dog flexor tendon anatomy is similar to humans[28], and they are large enough to perform an operative repair that is identical to that used in clinical practice. The canine flexor tendon repair model has been used over the past thirty years to investigate strategies for optimal suture repair[29], autogenic and allogenic graft repair[30], enhancing tendon-tendon or tendon-bone repair using

growth factors, cells and other therapeutics[31], and reducing tendon adhesions using biologic lubricants[32,33]. Furthermore, post-operative rehabilitation can be controlled in the canine model using a specially designed removable cast system which allows for replication of the controlled physical therapy that patients receive after tendon repair. The canine model has been used extensively to investigate the optimal rehabilitation parameters following flexor tendon repair[34].

Achilles Tendon Repair

Translational studies of Achilles tendon repair include those which investigate intra-tendinous repair as well as tendon-bone repair. The canine[35] and rabbit[36] have been used for translational studies of Achilles tendon repair using a variety of techniques and therapeutics for several decades. Their size allows for clinically relevant operative technique, and each model can be manipulated to control post-operative rehabilitation through casting and/or treadmill activity[37,38]. In more recent years, the rat model of Achilles tendon repair has been used extensively as well[39]. Although its size limits the use ofsome standard-of-care surgical techniques, post-operative loading of the repair can also be controlled through a variety of means such as casting, treadmill running or swimming in the rat model[40-42].

Rotator Cuff Repair

The rat model has been used most extensively to study the factors and strategies that influence rotator cuff repair[43].The rat's bony and muscle anatomy greatly resembles that of humans[44]. Re-tear of rotator cuff repairs has not been observed post-operatively in the rat model[45]. Hence, the rat model lends itself particularly well to studying regenerative (biologic) strategies for rotator cuff repair, but is a less suitable model for translational studies of

mechanically motivated standard-of-care repair techniques and strategies. The rat has been used to study the effect of post-operative activity levels[46] (see section below), chronic tears[47], and chronic tears followed by surgical repair[48]. Because chronic tendon tears in the rat are reparable through at least 16 weeks [48], the rat allows for studies of tendon-to-bone repair in the context of a clinically relevant chronic tendon injury, although in the absence of persistent degenerative muscle changes[49].

Large animals, such as the rabbit, dog, sheep and goat, have also been used to study surgical techniques[50] and regenerative strategies for rotator cuff repair, including using growth factors[51], scaffold interposition[52] and scaffold augmentation[53]. Because of their size, many standard-of-care surgical techniques can be reproduced in large animals. However, rotator cuff repairs in large animals uniformly undergo re-tear post-operatively[54-56], which confounds interpretation of the mechanical effectiveness of various repair strategies for the human condition. Further, the high incidence of tendon re-tear makes large animal models less suited to study biologic treatments aimed at tendon-to-bone healing because of the difficulty keeping the tendon and bone in close proximity after repair. The sheep and canine do not lend themselves to the study of chronic rotator cuff repair because chronically released rotator cuff tendons become irreparable after approximately 6 weeks[57,58]. Rabbit rotator cuff tendons, however, are reparable out to 12 weeks[59]. As a consequence of chronic tendon release, significant muscle atrophy and fatty infiltration develop and persist in large animal models[58,60,61] making them well-suited to study the mechanism and treatment of associated rotator cuff muscle pathology. A more exhaustive review of animal models for rotator cuff repair can be found elsewhere[62].

Tendon healing

Inflammation, proliferation, and remodeling in tendon healing

Tendon healing after surgical repair generally progresses through a short inflammatory phase, which lasts about a week, followed by a proliferative phase, which lastsa few weeks, followed by a remodeling phase, which lasts many months.[63]During the inflammatory phase, vascular permeability increases and an influx of inflammatory cells enter the healing site. These cells produce a number of cytokines and growth factors that lead to recruitment and proliferation of macrophages and resident tendon fibroblasts. During the proliferative and remodeling phases of healing, fibroblasts proliferate and begin to produce,deposit, orient, and crosslink fibrillar collagens.

Tendon healing generally involves the contributions of cells from multiple sources, including infiltrating inflammatory cells, resident fibroblasts fromthe tendon surface or midsubstance, and tendon or marrow-derived mesenchymal stem cells. Yet the specific cellular eventsin healing depend on the anatomy and physiology of a given tendon injury and repair. For example, healing of flexor tendon injuries begins with angiogenesis and epitenon fibroblast migration to the wound site.[64,65] Cells from the intrasynovial sheath infiltrate to the repair site, leading to adhesions between the sheath and the tendon surface, which impairs tendon gliding (and hence decreases digital range of motion).[65] In the rotator cuff, on the other hand, injuries typically require repair of tendon to bone. In this case, abundant fibroblasts from the tendon and surrounding tissues produce a disorganized collagen scar tissue at the attachment site of the two tissues.[46] Osteoclasts are also attracted to the repair site, and resorption of bone at the repair site can impair healing.[66] Understanding how different tendons heal is an important consideration for post-operative treatment and rehabilitation.

Recent evidence suggests that modulation of inflammation in the early stages following tendon repair may lead to improved healing.[67]It is important to recognize that regulated inflammation is largely beneficial to tissue repair, whereas excessive or persistence inflammation can be damaging. Indeed, whereas inflammatory cytokines attract fibroblasts to the repair site, excessive inflammation may lead to poor clinical outcomes.[68,69]Macrophages play essential roles in both promoting and resolving inflammation and in both facilitating and moderating tissue repair (Figure 1). That a single cell type can serve opposing functions may seem counterintuitive, but dramatic phenotypic changes occur when macrophages respond to local stimuli.[69,70] Macrophages are broadly classified into two groups, classically activated (M1) or alternatively activated (M2) cells, although it is important to note that many more phenotypes exist, each driven by specific activation conditions (Figure 1).[70]M1 macrophages, which are stimulated by bacterial products or Th1 cytokines, are pro-inflammatory (via release of IL1β, IL12, TNFα, others) and stimulate scarring and fibrosis. M2 macrophages, which are induced by Th2 cytokines, are anti-inflammatory (via release of IL10, TGFβ1, others) and are effective at clearing excessextracellular matrix (ECM) in scars. In an injury setting, M1 cells predominate early, whereas M2 macrophages accumulate later.[69]Ablation studies in liver, skin, and tendon show that during the early stages post-injury, macrophages (presumably M1) promote repair processes (i.e., re-epithelialization, myofibroblast activation, scarring, etc.) and inflammation, whereas at later stages, these cells (presumably M2) suppress inflammation and resolve scarring. Hence, in tendon injury, it would be reasonable to hypothesize that M1 macrophages promote repair by stimulating ECM production and that later on M2 macrophages repress inflammation and clear excess ECM, a concept that is consistent with experimental evidence.[68]Disturbing the balance between these macrophage subtypes may result in defective repair and impaired tissue

function. For example, over-activation or an abundance of M1 macrophages could lead to deleterious inflammation and excess ECM production, whereas sustained or an excess of M2 cells could cause excess tissue remodeling resulting in tissue damage. Thus, understanding the signals that control macrophageactivation will provide fundamental insights to how tissue repair processes are orchestrated and balanced.

A number of growth factors, powerful regulators of biological function, play important roles during the remodeling phase of tendon healing.[71]The patterns of natural expression of platelet derived growth factor (PDGF-BB), basic fibroblast growth factor (bFGF), transforming growth factor β (TGF-β), and vascular endothelial growth factor (VEGF) vary dramatically over time during tendon healing. Manipulation of the growth factor environment has therefore been an important strategy for improving the outcomes of repaired tendon and ligament.[32,72]PDGF and bFGF have been effective in promoting fibroblast proliferation and collagen remodeling;however, bFGF has also been shown to promote adhesions in a flexor tendon animal model.[32,73] TGF-β has received particular attention in the tendon literature due to its critical role in tendon developmentand its potent affect in promoting matrix remodeling.[74] Furthermore, fetal tendonwounds have been shown to heal in a regenerative manner (i.e., the repaired tissue is identical to the original tissue) and this process may beregulated by TGF-β isoforms.[75,76]Specifically, regenerative fetal wound healing was characterized by low expression of TGF-β1 and TGF-β2 and high expression of TGF-β3. In contrast, adult scar-mediated wound healing was characterized by high levels of TGF-β1 and TGF-β2 and low levels of TGF-β3. However, therapeutic application of this concept has not to date beensuccessful, as control of TGF-β isoforms during tendon-to-bone healing in a rat rotator cuff model did not lead to regenerative healing.[77] These initial therapeutic studies using growth factors to improve tendon

healingdemonstratethat dosage, time of administration, residence time and synergistic effects significantly complicate the use of growth factors as a treatment strategy.

Rehabilitation strategies for enhanced tendon healing

Tendon development, homeostasis, and healing are influenced by their loading environments.[78] Muscle loading is necessary for tendon development and maintenance of adult tendon mass and mechanical properties. The effects of loading on healing tendons, however, are complex. Optimal post-repair rehabilitation strategies for tendon depend on the particular tendon's environment and functional requirements. For example, successful repair of flexor tendonsrequires both gliding and strength for digital function. Immobilization after repair of flexor tendons leads to adhesions between the tendon and its synovial sheath, limiting tendon excursion and hence decreasing finger range of motion and tendon strength.[79]Passive motion rehabilitation, on the other hand, has been shown in animal models and clinical practice to greatly improve post-repair function, leading to improved tendon gliding and increased repair strength compared to both immobilization and active force rehabilitation.[80]

In contrast, studies in the rat rotator cuff model have suggested a beneficial effect of immobilization to prevent post-repair gapping and aid in healing.[46] Protective immobilization was shown to improve healing compared to other post-repair loading protocolssuch exercise or complete tendon unloading.[46]The mechanisms behind the benefits of immobilization are unclear, but likely include mechanical (i.e., prevention of gap formation) and biologic effects(e.g., reduced phagocytic macrophage accumulation[81]).Recent evidence that rotator cuff re-tears in human patients occur within the first 3-6 months post-operation[82,83] supports a conservative

approach to rehabilitation after repair, though to date no apparent advantage or disadvantage of shoulder immobilization compared with early passive range of motion has been shown.[84]

In summary, rehabilitation strategies must balance the negative outcomes that can arise from immobilization (e.g., increased adhesions,retarded repair tissue maturation, joint stiffness)with the negative outcomes that can arise from too much load (e.g.,repair tissue rupture)(Figure 2). Furthermore, the particular anatomy and functional requirement of a giventendon repairmust be considered when determining the optimal rehabilitation scenario.

Conclusions and open questions

Treatment of tendon injuries is a significant clinical challenge. The basic science of intra-tendinous and tendon-bone healing remainsonly partially understood. Over the past three decades, advances have been made in the treatment of certain tendinopathies by first understanding injury and healing in animal models and then translating that understanding to clinical care. Despite these advances, a number of open questions remain, including:

- Does it matter which injury mechanism and/or tendon we use to explore the basic science of tendon healing?
- What is the"Goldilocks balance" for macrophage response (e.g., M1 vs. M2), rehabilitation (e.g., immobilization vs. loading), and other mechanisms that influence repair?
- What are the appropriate animal models for basic and translational questions related to various tendon injury and repair scenarios?
- How can we harness the power of growth factors to improve tendon repair?
- Are there translational questions that cannot be answered in animal models?

- How does physical therapy translate to cell-level responses?

Acknowledgements

Support from the National Institutes of Health (AR057836, AR060820).

References

1. de Jong JP, Nguyen JT, Sonnema AJ, et al. 2014. The incidence of acute traumatic tendon injuries in the hand and wrist: a 10-year population-based study. Clinics in orthopedic surgery 6:196-202.
2. Jarvinen TA, Kannus P, Maffulli N, et al. 2005. Achilles tendon disorders: etiology and epidemiology. Foot and ankle clinics 10:255-266.
3. Raikin SM, Garras DN, Krapchev PV. 2013. Achilles tendon injuries in a United States population. Foot & ankle international / American Orthopaedic Foot and Ankle Society [and] Swiss Foot and Ankle Society 34:475-480.
4. Astrom M, Rausing A. 1995. Chronic Achilles tendinopathy. A survey of surgical and histopathologic findings. Clin Orthop Relat Res (316):151-164.
5. Tashjian RZ. 2012. Epidemiology, natural history, and indications for treatment of rotator cuff tears. Clin Sports Med 31:589-604.
6. Tempelhof S. 1999. Age-related prevalance of rotator cuff tears in asymptomatic shoulders. J Shoulder Elbow Surg 8:296-299.

7. Longo UG, Franceschi F, Ruzzini L, et al. 2008. Histopathology of the supraspinatus tendon in rotator cuff tears. Am J Sports Med 36:533-538.
8. Goodship AE, Birch HL, Wilson AM. 1994. The pathobiology and repair of tendon and ligament injury. The Veterinary clinics of North America Equine practice 10:323-349.
9. Soslowsky LJ, Thomopoulos S, Tun S, et al. 2000. Neer Award 1999. Overuse activity injures the supraspinatus tendon in an animal model: a histologic and biomechanical study. J Shoulder Elbow Surg 9:79-84.
10. Pingel J, Wienecke J, Kongsgaard M, et al. 2013. Increased mast cell numbers in a calcaneal tendon overuse model. Scand J Med Sci Sports 23:e353-360.
11. Andarawis-Puri N, Sereysky JB, Sun HB, et al. 2012. Molecular response of the patellar tendon to fatigue loading explained in the context of the initial induced damage and number of fatigue loading cycles. J Orthop Res 30:1327-1334.
12. Sereysky JB, Andarawis-Puri N, Jepsen KJ, et al. 2012. Structural and mechanical effects of in vivo fatigue damage induction on murine tendon. J Orthop Res 30:965-972.
13. Smith MM, Sakurai G, Smith SM, et al. 2008. Modulation of aggrecan and ADAMTS expression in ovine tendinopathy induced by altered strain. Arthritis Rheum 58:1055-1066.
14. Solchaga LA, Bendele A, Shah V, et al. 2014. Comparison of the effect of intra-tendon applications of recombinant human platelet-derived growth factor-BB, platelet-rich plasma, steroids in a rat achilles tendon collagenase model. J Orthop Res 32:145-150.
15. Chang KV, Wu CH, Ding YH, et al. 2012. Application of contrast-enhanced sonography with time-intensity curve analysis to explore hypervascularity in Achilles tendinopathy

by using a rabbit model. Journal of ultrasound in medicine : official journal of the American Institute of Ultrasound in Medicine 31:737-746.

16. Lacitignola L, Staffieri F, Rossi G, et al. 2014. Survival of bone marrow mesenchymal stem cells labelled with red fluorescent protein in an ovine model of collagenase-induced tendinitis. Veterinary and comparative orthopaedics and traumatology : VCOT 27:204-209.
17. Guerquin MJ, Charvet B, Nourissat G, et al. 2013. Transcription factor EGR1 directs tendon differentiation and promotes tendon repair. J Clin Invest 123:3564-3576.
18. Schizas N, Li J, Andersson T, et al. 2010. Compression therapy promotes proliferative repair during rat Achilles tendon immobilization. J Orthop Res 28:852-858.
19. Beason DP, Kuntz AF, Hsu JE, et al. 2012. Development and evaluation of multiple tendon injury models in the mouse. J Biomech 45:1550-1553.
20. O'Brien EJ, Frank CB, Shrive NG, et al. 2012. Heterotopic mineralization (ossification or calcification) in tendinopathy or following surgical tendon trauma. Int J Exp Pathol 93:319-331.
21. Freedman BR, Sarver JJ, Buckley MR, et al. 2014. Biomechanical and structural response of healing Achilles tendon to fatigue loading following acute injury. J Biomech 47:2028-2034.
22. Adams SB, Jr., Thorpe MA, Parks BG, et al. 2014. Stem cell-bearing suture improves Achilles tendon healing in a rat model. Foot & ankle international / American Orthopaedic Foot and Ankle Society [and] Swiss Foot and Ankle Society 35:293-299.

23. Galatz LM, Sandell LJ, Rothermich SY, et al. 2006. Characteristics of the rat supraspinatus tendon during tendon-to-bone healing after acute injury. J Orthop Res 24:541-550.

24. Fujioka H, Thakur R, Wang GJ, et al. 1998. Comparison of surgically attached and non-attached repair of the rat Achilles tendon-bone interface. Cellular organization and type X collagen expression. Connect Tissue Res 37:205-218.

25. Loiselle AE, Bragdon GA, Jacobson JA, et al. 2009. Remodeling of murine intrasynovial tendon adhesions following injury: MMP and neotendon gene expression. J Orthop Res 27:833-840.

26. Kobayashi M, Itoi E, Minagawa H, et al. 2006. Expression of growth factors in the early phase of supraspinatus tendon healing in rabbits. J Shoulder Elbow Surg 15:371-377.

27. Chen JW, Galloway JL. 2014. The development of zebrafish tendon and ligament progenitors. Development 141:2035-2045.

28. Potenza AD. 1962. Tendon healing within the flexor digital sheath in the dog. J Bone Joint Surg Am 44-A:49-64.

29. Winters SC, Gelberman RH, Woo SL, et al. 1998. The effects of multiple-strand suture methods on the strength and excursion of repaired intrasynovial flexor tendons: a biomechanical study in dogs. J Hand Surg Am 23:97-104.

30. Gelberman RH, Chu CR, Williams CS, et al. 1992. Angiogenesis in healing autogenous flexor-tendon grafts. J Bone Joint Surg Am 74:1207-1216.

31. Zhao C, Ozasa Y, Reisdorf RL, et al. 2014. CORR(R) ORS Richard A. Brand Award for Outstanding Orthopaedic Research: Engineering flexor tendon repair with lubricant, cells, and cytokines in a canine model. Clin Orthop Relat Res 472:2569-2578.

32. Thomopoulos S, Das R, Silva MJ, et al. 2009. Enhanced flexor tendon healing through controlled delivery of PDGF-BB. J Orthop Res 27:1209-1215.

33. Zhao C, Hashimoto T, Kirk RL, et al. 2013. Resurfacing with chemically modified hyaluronic acid and lubricin for flexor tendon reconstruction. J Orthop Res 31:969-975.

34. Silva MJ, Brodt MD, Boyer MI, et al. 1999. Effects of increased in vivo excursion on digital range of motion and tendon strength following flexor tendon repair. J Orthop Res 17:777-783.

35. Gilbert TW, Stewart-Akers AM, Simmons-Byrd A, et al. 2007. Degradation and remodeling of small intestinal submucosa in canine Achilles tendon repair. J Bone Joint Surg Am 89:621-630.

36. Deng D, Wang W, Wang B, et al. 2014. Repair of Achilles tendon defect with autologous ASCs engineered tendon in a rabbit model. Biomaterials 35:8801-8809.

37. Nielsen C, Pluhar GE. 2006. Outcome following surgical repair of achilles tendon rupture and comparison between postoperative tibiotarsal immobilization methods in dogs: 28 cases (1997-2004). Veterinary and comparative orthopaedics and traumatology : VCOT 19:246-249.

38. West JR, Juncosa N, Galloway MT, et al. 2004. Characterization of in vivo Achilles tendon forces in rabbits during treadmill locomotion at varying speeds and inclinations. J Biomech 37:1647-1653.

39. Kraus TM, Imhoff FB, Wexel G, et al. 2014. Stem cells and basic fibroblast growth factor failed to improve tendon healing: an in vivo study using lentiviral gene transfer in a rat model. J Bone Joint Surg Am 96:761-769.

40. Murrell GA, Jang D, Deng XH, et al. 1998. Effects of exercise on Achilles tendon healing in a rat model. Foot & ankle international / American Orthopaedic Foot and Ankle Society [and] Swiss Foot and Ankle Society 19:598-603.

41. Bring D, Reno C, Renstrom P, et al. 2010. Prolonged immobilization compromises up-regulation of repair genes after tendon rupture in a rat model. Scand J Med Sci Sports 20:411-417.

42. Karpakka J, Vaananen K, Virtanen P, et al. 1990. The effects of remobilization and exercise on collagen biosynthesis in rat tendon. Acta Physiol Scand 139:139-145.

43. Thomopoulos S, Soslowsky LJ, Flanagan CL, et al. 2002. The effect of fibrin clot on healing rat supraspinatus tendon defects. J Shoulder Elbow Surg 11:239-247.

44. Soslowsky LJ, Carpenter JE, DeBano CM, et al. 1996. Development and use of an animal model for investigations on rotator cuff disease. J Shoulder Elbow Surg 5:383-392.

45. Galatz LM, Charlton N, Das R, et al. 2009. Complete removal of load is detrimental to rotator cuff healing. Journal of Shoulder and Elbow Surgery 18:669-675.

46. Thomopoulos S, Williams GR, Soslowsky LJ. 2003. Tendon to bone healing: differences in biomechanical, structural, and compositional properties due to a range of activity levels. Journal of Biomechanical Engineering 125:106-113.

47. Gimbel JA, Van Kleunen JP, Mehta S, et al. 2004. Supraspinatus tendon organizational and mechanical properties in a chronic rotator cuff tear animal model. J Biomech 37:739-749.

48. Gimbel JA, Van Kleunen JP, Lake SP, et al. 2007. The role of repair tension on tendon to bone healing in an animal model of chronic rotator cuff tears. J Biomech 40:561-568.

49. Barton ER, Gimbel JA, Williams GR, et al. 2005. Rat supraspinatus muscle atrophy after tendon detachment. J Orthop Res 23:259-265.

50. Klinger HM, Buchhorn GH, Heidrich G, et al. 2008. Biomechanical evaluation of rotator cuff repairs in a sheep model: suture anchors using arthroscopic Mason-Allen stitches compared with transosseous sutures using traditional modified Mason-Allen stitches. Clin Biomech (Bristol, Avon) 23:291-298.

51. Seeherman HJ, Archambault JM, Rodeo SA, et al. 2008. rhBMP-12 accelerates healing of rotator cuff repairs in a sheep model. J Bone Joint Surg Am 90:2206-2219.

52. Adams JE, Zobitz ME, Reach JS, Jr., et al. 2006. Rotator cuff repair using an acellular dermal matrix graft: an in vivo study in a canine model. Arthroscopy 22:700-709.

53. Derwin KA, Codsi MJ, Milks RA, et al. 2009. Rotator cuff repair augmentation in a canine model with use of a woven poly-L-lactide device. J Bone Joint Surg Am 91:1159-1171.

54. Derwin KA, Baker AR, Codsi MJ, et al. 2007. Assessment of the canine model of rotator cuff injury and repair. J Shoulder Elbow Surg 16:S140-148.

55. Rodeo SA, Potter HG, Kawamura S, et al. 2007. Biologic augmentation of rotator cuff tendon-healing with use of a mixture of osteoinductive growth factors. J Bone Joint Surg Am 89:2485-2497.

56. Schlegel TF, Hawkins RJ, Lewis CW, et al. 2007. An in vivo comparison of the modified Mason-Allen suture technique versus an inclined horizontal mattress suture technique with regard to tendon-to-bone healing: a biomechanical and histologic study in sheep. J Shoulder Elbow Surg 16:115-121.

57. Coleman SH, Fealy S, Ehteshami JR, et al. 2003. Chronic rotator cuff injury and repair model in sheep. J Bone Joint Surg Am 85-A:2391-2402.

58. Safran O, Derwin KA, Powell K, et al. 2005. Changes in rotator cuff muscle volume, fat content, and passive mechanics after chronic detachment in a canine model. J Bone Joint Surg Am 87:2662-2670.

59. Koike Y, Trudel G, Curran D, et al. 2006. Delay of supraspinatus repair by up to 12 weeks does not impair enthesis formation: A quantitative histologic study in rabbits. Journal of orthopaedic research : official publication of the Orthopaedic Research Society 24:202-210.

60. Gerber C, Meyer DC, Schneeberger AG, et al. 2004. Effect of tendon release and delayed repair on the structure of the muscles of the rotator cuff: an experimental study in sheep. J Bone Joint Surg Am 86-A:1973-1982.

61. Uhthoff HK, Matsumoto F, Trudel G, et al. 2003. Early reattachment does not reverse atrophy and fat accumulation of the supraspinatus--an experimental study in rabbits. Journal of orthopaedic research : official publication of the Orthopaedic Research Society 21:386-392.

62. Derwin KA, Baker AR, Iannotti JP, et al. 2010. Preclinical models for translating regenerative medicine therapies for rotator cuff repair. Tissue Eng Part B Rev 16:21-30.

63. Voleti PB, Buckley MR, Soslowsky LJ. 2012. Tendon healing: repair and regeneration. Annu Rev Biomed Eng 14:47-71.

64. Manning CN, Havlioglu N, Knutsen E, et al. 2014. The early inflammatory response after flexor tendon healing: a gene expression and histological analysis. J Orthop Res 32:645-652.

65. Gelberman RH, Vandeberg JS, Manske PR, et al. 1985. The early stages of flexor tendon healing: a morphologic study of the first fourteen days. J Hand Surg Am 10:776-784.

66. Ditsios K, Boyer MI, Kusano N, et al. 2003. Bone loss following tendon laceration, repair and passive mobilization. J Orthop Res 21:990-996.

67. Hays PL, Kawamura S, Deng XH, et al. 2008. The role of macrophages in early healing of a tendon graft in a bone tunnel. J Bone Joint Surg Am 90:565-579.

68. Sugg KB, Lubardic J, Gumucio JP, et al. 2014. Changes in macrophage phenotype and induction of epithelial-to-mesenchymal transition genes following acute Achilles tenotomy and repair. J Orthop Res 32:944-951.

69. Lichtnekert J, Kawakami T, Parks WC, et al. 2013. Changes in macrophage phenotype as the immune response evolves. Curr Opin Pharmacol 13:555-564.

70. Murray PJ, Allen JE, Biswas SK, et al. 2014. Macrophage activation and polarization: nomenclature and experimental guidelines. Immunity 41:14-20.

71. Molloy T, Wang Y, Murrell G. 2003. The roles of growth factors in tendon and ligament healing. Sports Medicine 33:381-394.

72. Spindler KP, Dawson JM, Stahlman GC, et al. 2002. Collagen expression and biomechanical response to human recombinant transforming growth factor beta (rhTGF-beta2) in the healing rabbit MCL. Journal of Orthopaedic Research 20:318-324.

73. Thomopoulos S, Kim HM, Das R, et al. 2010. The effects of exogenous basic fibroblast growth factor on intrasynovial flexor tendon healing in a canine model. J Bone Joint Surg Am 92:2285-2293.

74. Glass ZA, Schiele NR, Kuo CK. 2014. Informing tendon tissue engineering with embryonic development. J Biomech 47:1964-1968.

75. Shah M, Foreman DM, Ferguson MW. 1995. Neutralisation of TGF-beta 1 and TGF-beta 2 or exogenous addition of TGF-beta 3 to cutaneous rat wounds reduces scarring. Journal of cell science 108 (Pt 3):985-1002.

76. Beredjiklian PK, Favata M, Cartmell JS, et al. 2003. Regenerative versus reparative healing in tendon: a study of biomechanical and histological properties in fetal sheep. Ann Biomed Eng 31:1143-1152.

77. Kim HM, Galatz LM, Das R, et al. 2011. The role of transforming growth factor beta isoforms in tendon-to-bone healing. Connect Tissue Res Epub.

78. Killian ML, Cavinatto L, Galatz LM, et al. 2012. The role of mechanobiology in tendon healing. J Shoulder Elbow Surg 21:228-237.

79. Woo SL, Gelberman RH, Cobb NG, et al. 1981. The importance of controlled passive mobilization on flexor tendon healing. A biomechanical study. Acta Orthop Scand 52:615-622.

80. Boyer MI, Goldfarb CA, Gelberman RH. 2005. Recent progress in flexor tendon healing. The modulation of tendon healing with rehabilitation variables. J Hand Ther 18:80-85; quiz 86.

81. Dagher E, Hays PL, Kawamura S, et al. 2009. Immobilization modulates macrophage accumulation in tendon-bone healing. Clin Orthop Relat Res 467:281-287.

82. Miller BS, Downie BK, Kohen RB, et al. 2011. When do rotator cuff repairs fail? Serial ultrasound examination after arthroscopic repair of large and massive rotator cuff tears. Am J Sports Med 39:2064-2070.

83. Iannotti JP, Deutsch A, Green A, et al. 2013. Time to failure after rotator cuff repair: a prospective imaging study. J Bone Joint Surg Am 95:965-971.

84. Keener JD, Galatz LM, Stobbs-Cucchi G, et al. 2014. Rehabilitation following arthroscopic rotator cuff repair: a prospective randomized trial of immobilization compared with early motion. J Bone Joint Surg Am 96:11-19.

Tables

Table 1: Animal models for studying tendon healing.

Research Question	Animal Models
Basic Mechanisms of Chronic Tendon Injury	Models of Inducing Chronic Tendon Injury (Mouse, Rat, Rabbit, Sheep) • Uphill or downhill treadmill running • Controlled fatigue loading under anesthesia • Partial laceration • Collagenase injection
Basic Mechanisms of Tendon Healing	Models of Tendon Healing (Mouse, Rat, Rabbit) A) Intra-tendinous healing following injury induced by: • Sharp or blunt transection • Punch biopsy or window defect • Needle stick • Collagenase injection B) Tendon-bone healing following sharp transection and suture repair to bone
Translation to Clinical Care	Translational Models of Tendon Injury and Repair • Flexor Tendon: Canine • Achilles Tendon: Rat, Rabbit, Canine • Rotator Cuff Tendon: Rat, Rabbit, Canine, Ovine

Figure Legends

Figure 1:(a) Macrophages can differentiate into specific subpopulations with distinct phenotypes and functions. **(b)**In acute inflammation, macrophage phenotypes such as M2 and Mreg are usually beneficial for immunosuppression, scar resolution, and remodeling. In chronic inflammation, macrophage phenotypes such as M2 and M2a can stimulate excessive tissue remodeling resulting in fibrosis. [Reproduced, with permission, from [69]]

Figure2: To achieve effective healing, a balance must be reached betweenloads that are too low (leading to increased adhesions, retarded repair tissue maturation, and/or joint stiffness) and loads that are too high (leading to repair site gapping or rupture).[78]

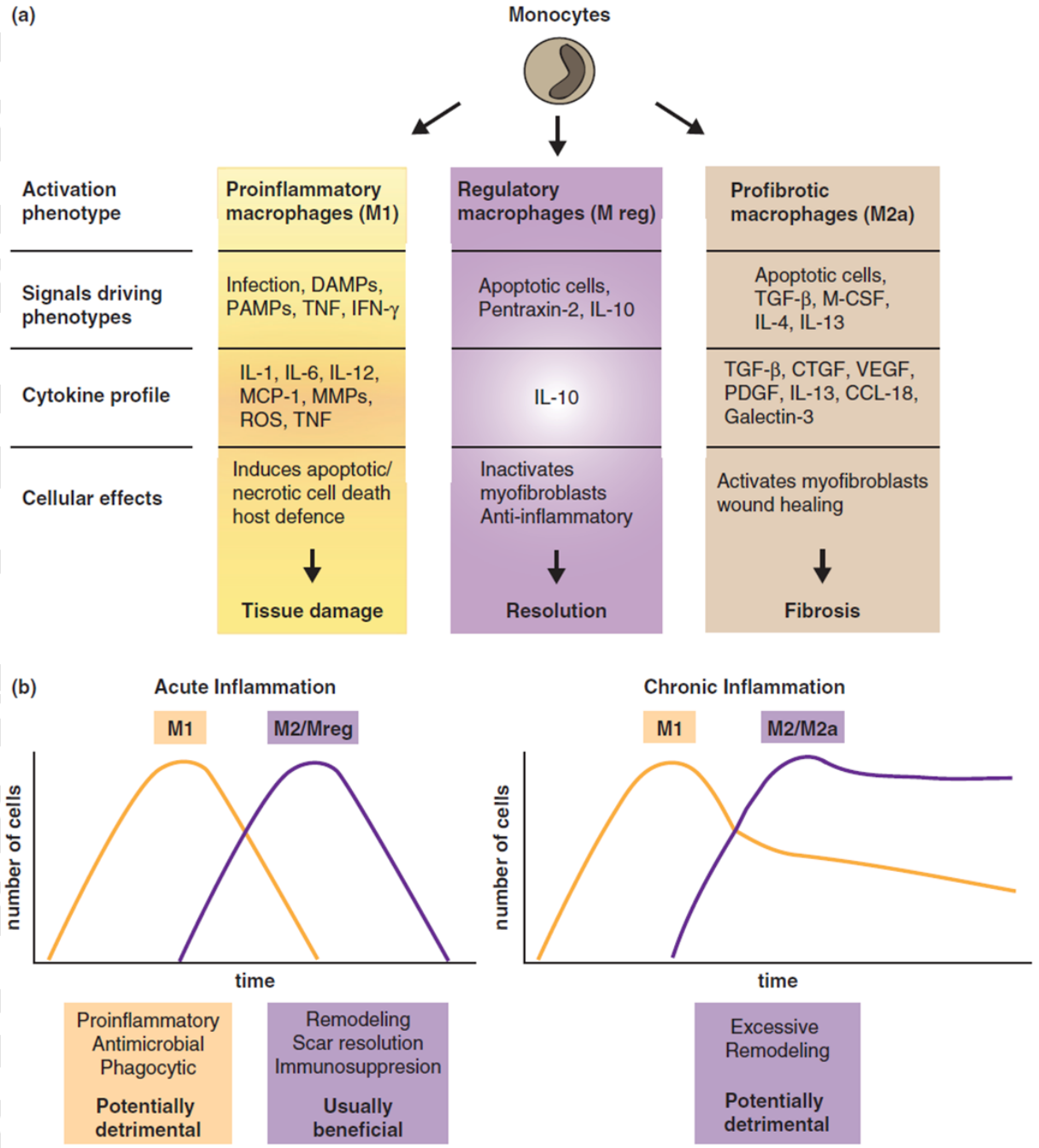

Figure 1

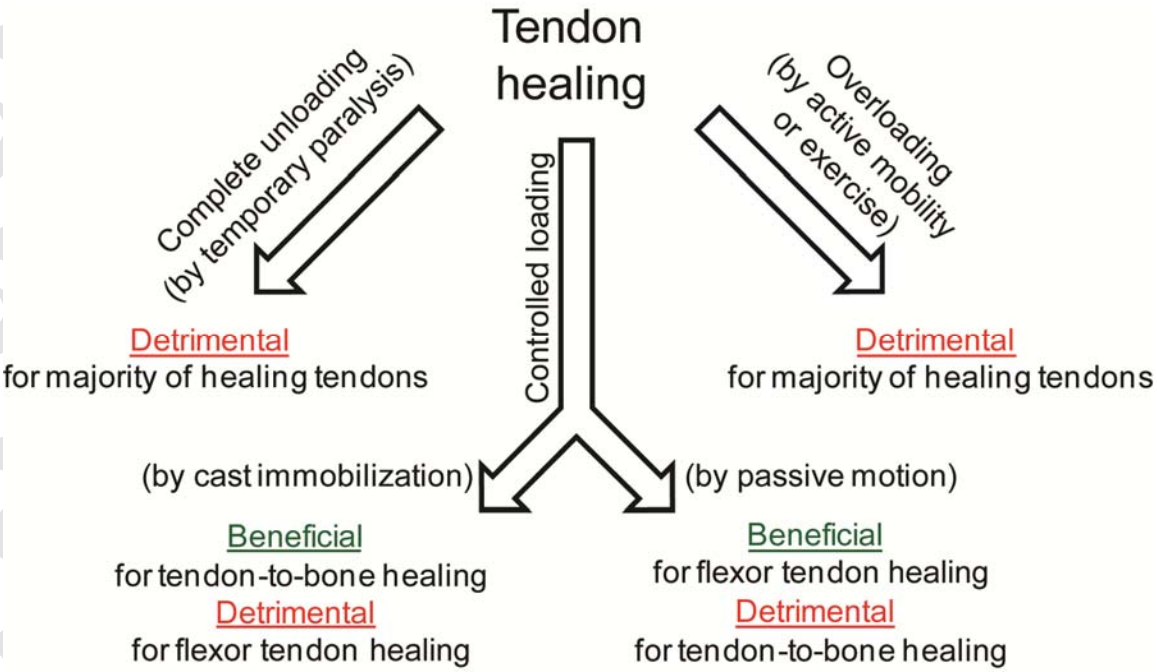

Figure 2