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ORIGINAL ARTICLE

Generation of a new model of patellar tendinopathy in rats which mimics the human sports pathology: A pilot study

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KEYWORDS
Tendon;
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Abstract
Introduction: Patellar tendon pathophysiology is not still fully understood. The collection of clinical samples from athletes that could permit the analysis of the tendinopathy progression, especially in the early stages, is difficult. For that reason, the purpose of this study is to develop a new experimental animal model of patellar tendinopathy in rats which mimics the human tendinopathy by in vivo intratendinous collagenase injection in the proximal portion of the patellar tendon.
Material and methods: The experimental model used was 8-week-old male Wistar rats ($N = 4$). The administration of collagenase was performed by ultrasound-guided puncture at the level of the proximal and deep portion of the patellar tendon in anesthetized animals. The tendon lesion was evaluated 48 h after injury by magnetic resonance and then, the animals were euthanized and the patellar tendons were collected for histological evaluation.
Results: The collagenase-induced lesion model demonstrated important similarities with the human patellar tendinopathy in the region of the proximal insertion.

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Conclusions: The experimental model of patellar tendinopathy in rat model induces a degeneration and distortion of the patellar tendon architecture in its proximal portion, which closely mimics to that seen in human patellar tendinopathy, and could represent an excellent preclinical model for the study of new therapies focused on treatment of tendinopathy.

PALABRAS CLAVE
Tendón;
Modelo;
Colagenasa

Generación de un nuevo modelo de tendinopatía rotuliana en ratas que imita la patología deportiva humana: estudio piloto

Resumen
Introducción: La fisiopatología de la tendinopatía rotuliana no es del todo conocida. La obtención de muestras clínicas en deportistas que permitan conocer la historia natural de la tendinopatía, sobre todo en las primeras etapas, es difícil. Por este motivo, el propósito de este estudio es crear, en una primera fase, un modelo experimental de tendinopatía rotuliana que simule la tendinopatía humana mediante la aplicación de colagenasa en tendón rotuliano en modelo de rata.
Material y métodos: El modelo experimental utilizado fueron ratas Wistar macho de 8 semanas de edad (N = 4). La administración de colagenasa se realizó, tras anestesia e inmovilización de los animales, mediante punción guiada por ecografía a nivel de la porción proximal y profunda del tendón rotuliano. La lesión tendinosa se evaluó 48 h después de la lesión mediante RMN tras lo cual se procedió a la eutanasia de los animales y extracción de los tendones rotulianos para su evaluación histológica.
Resultados: El modelo de lesión inducida con colagenasa demostró similitud a nivel de la histología con la tendinopatía rotuliana humana en la región de su inserción proximal.
Conclusiones: El modelo experimental de tendinopatía rotuliana en ratas induce la degeneración y distorsión de la arquitectura del tendón rotuliano en su porción proximal, situación similar a la observada en la tendinopatía rotuliana humana, y representa un excelente modelo preclínico para el estudio de nuevas terapias enfocadas al tratamiento de la tendinopatía.

Introduction

Patellar tendinopathy was first described by Blazina in 1973 as the ''jumper's knee''.[1] It is a frequent pathology affecting the proximal insertion of the patellar tendon and is one of the most common forms of chronic tendinopathy in athletes.[2] Although numerous theories have been proposed, the causes and pathophysiology of patellar tendinopathy are still poorly understood.[3,4]

Cook and Purdam[5] proposed a model based on the continuum of tendon pathology. This model considers 3 phases which may overlap and interconnect over time: reactive tendinopathy, tendon deterioration (failure of the healing process) and degenerative tendinopathy. This model proposes that the work load on the tendon plays an essential role in the onset and progression of tendinopathy.[5] The findings commonly found in athletes are a degenerative tendinopathy, characterized by an increase in the non-collagen matrix (proteoglycans), mucoid degeneration with variable fibrosis, neovascularization, and an increase in the cellularity due to fibroblasts.[6] On the other hand, Fu et al.[7] also proposed a three-stage model characterized by an initial injury, a healing process and a failed clinical presentation. Despite the clinical importance of tendinopathy, its pathophysiology and clinical evolution are still poorly understood, which restricts the therapeutic interventions. The advance in the knowledge and understanding of the clinical evolution of tendinopathy presents important limitations, not only in the hitches to obtain tissue samples from human athletes but also due to these samples represent only the final stages of pathological processes with undisclosed onset and duration.[8–10] For this reason, several animal models have been proposed for the study of tendinopathy, although their similarities with the human tendinopathy and their suitability to study the patellar tendon pathophysiology are still under controversy.[11–13]

Several animal models have been described in order to study the patellar tendinopathy, such as the surgically-induced tendon injuries,[14–22] intratendinous collagenase injection,[23–31] by administration of biological substances[32,33] or induced by overuse,[34–36] however, most of them are used for the testing of therapeutic approaches and only few are utilized to elucidate the pathological mechanisms of patellar tendinopathy. For this reason, the present work proposes

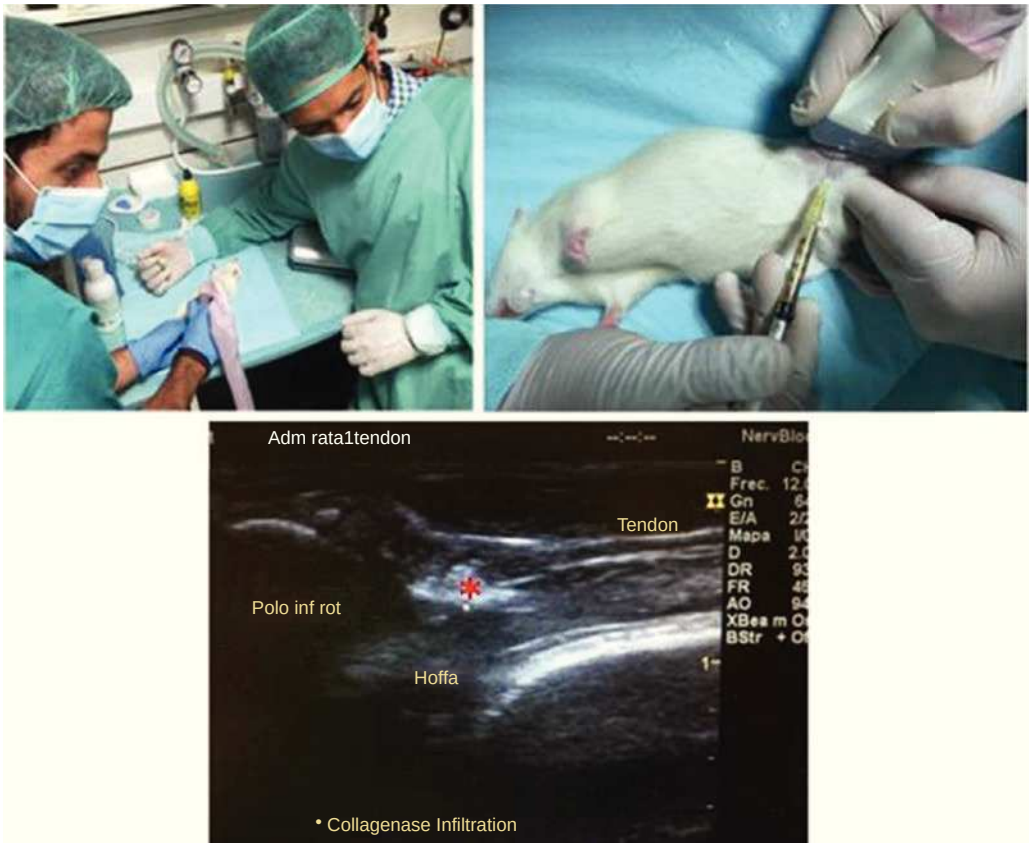

Figure 1 Collagenase-based injury model. A dose of collagenase was administered in the proximal and deep portion of the patellar tendon by ultrasound-guided injection (*).

the generation of a new animal model able to reproduce the patellar tendinopathy of the deep proximal portion that is commonly observed in athletes. This model could allow us to study and understand the pathological evolution of tendinopathy in its proximal portion, as well as to evaluate the effectiveness of new therapeutic interventions in order to promote the healing processes of patellar tendon.

Materials and methods

Animals

The experimental animal model used was 8-week-old male Wistar rats (Harlan). The total number of animals used was 4. The rats were maintained at 22–24 °C in a dark/light cycle of 12 h, with access to food and water ad libitum. All procedures were carried out in accordance with Spanish (Real Decreto 53/2013) and European (2010/63/EU) legislation and approved by the Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural del Gobierno Catalán (Generalitat de Catalunya).

Collagenase-based injury model

Animals were anesthetized by intraperitoneal injection of a mixture of ketamine (75 mg/kg) and xylazine (10 mg/kg) and placed in supine position facing up the left knee joint prior to the surgical procedures. The surgical process for the generation of patellar tendon injury is illustrated in Fig. 1. Knee joint was previously shaved and sterilized with 70% alcohol. The tendon injury was generated by ultrasound-guided injection of 10% collagenase solution dissolved in saline previously filtered for sterilization with a 0.22 μm filter (Nalgene), using a 29 G needle (0.33 mm inner diameter). The intervention was performed on the anterior side of the knee, inserting the needle into the proximal portion of the tendon until reaching the deep fibers. Once in the selected area, 20 μl of collagenase solution were released in the proximal tendon insertion area (Fig. 1). After the surgical procedure, post-surgical analgesia (buprenorphine 0.01 mg/kg) was administered subcutaneously to all animals. Our study followed the ethics standards in sport and research in sport sciences.[37]

Magnetic resonance imaging analysis

In vivo ^{1}H-magnetic resonance imaging (MRI) studies were performed at the Autonomous University of Barcelona (UAB, Barcelona, Spain) using a 7T Bruker BioSpec 70/30 USR (Bruker BioSpin GmbH, Ettlingen, Germany) system equipped with a mini-imaging gradient set (400mT/m) and using a quadrature transceiver volume coil with 72 mm inner diameter.

Rats were positioned in a bed, which allowed delivery of anesthesia (isoflurane, 1.5–2.0% in O_2 at 1 L/min),

Figure 2 Anestesized animals were monitored for temperature and respiratory frequency during MRI analysis. High resolution T2-weighted fast spin-echo sagittal images were acquired of the injured and contralateral patellar tendons.

with an integrated heat water circuit for body temperature regulation (Fig. 2). Body temperature was measured with a rectal probe and maintained at $37 \pm 1\,^{\circ}C$. Respiratory frequency was monitored with a pressure probe and kept between 60 and 80 breaths/min. Low resolution T2-weighted fast spin-echo images were initially obtained in axial, sagittal and coronal planes to be used as reference scout images. Imaging parameters for these images were: effective echo time (TEeff) = 36 ms; repetition time (TR) = 3 s; echo train length (ETL) = 8; field of view (FOV) = $6 \times 6\,cm^2$; matrix size (MTX) = 128×128; slice thickness (ST) = 2 mm; gap between slices (gap) = 0.5 mm; number of slices (NS) = 25 – axial, 10 – sagittal, 11 – coronal; number of averages (NA) = 1. High resolution T2-weighted fast spin-echo images were acquired afterwards in sagittal planes containing the lesion and the contralateral side. Experimental parameters for these images were: TEeff = 30 ms; TR = 4 s; ETL = 8; FOV = $3.2 \times 3.2\,cm^2$; MTX = 256×256; ST = 1 mm; gap = 0.1 mm; NS = 18; NA = 12; experimental time = 25 min 36 s. MRI data were acquired and processed on a Linux computer using Paravision 5.1 software (Bruker BioSpin GmbH, Ettlingen, Germany).

Histological analysis

After 7 days post-injury, the animals were euthanized by an intraperitoneal administration of ketamine (75 mg/kg) and xylazine (10 mg/kg) overdose. Tendons from both legs were immediately extracted from the patella to the tibia insertion including the Hoffa fat pad. Specimens were frozen in 2-methylbutane (Alfa Aesar, Johnson Matthey Company, Karlsruhe, Germany) previously supercooled in liquid nitrogen, and stored at $-80\,^{\circ}C$ until used. The tendon samples were sectioned longitudinally (10 μm thick) using a cryotome (Leica Microsystems, Wetzlar, Germany) at $-20\,^{\circ}C$ and mounted on Polylisine™ slides (VWR, Leuven, Belgium). Consecutive frozen tendon

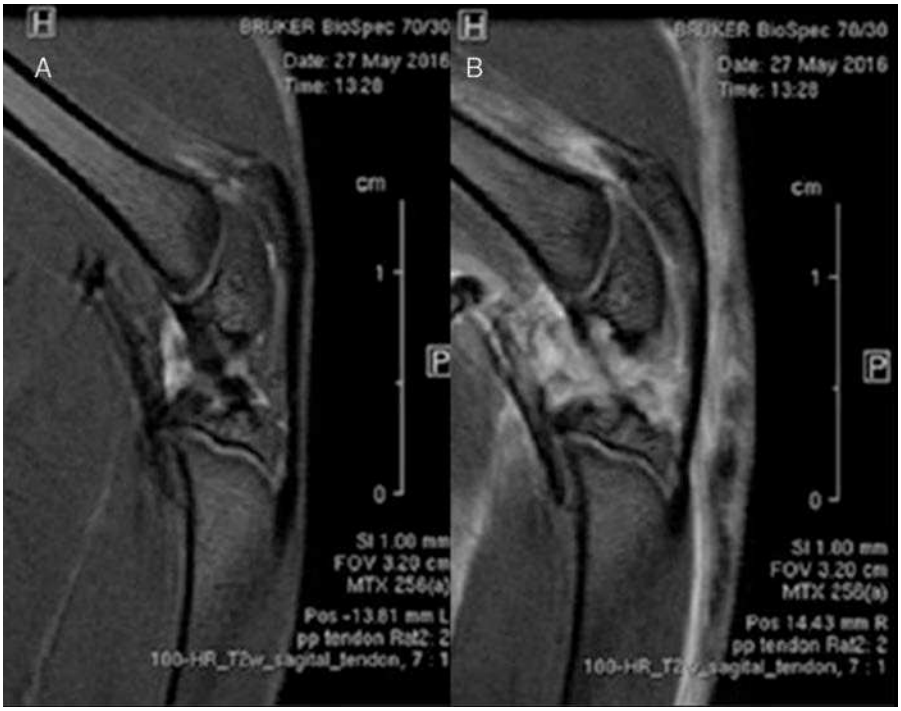

Figure 3 Comparative study using magnetic resonance imaging. No differences were found in the structure of the healthy patellar tendon (A) and in the injured tendon (B).

sections were used for histological analysis. Samples were stained with hematoxylin-eosin (1 min hematoxylin and 15 s eosin), washed in water and dehydrated with ethanol gradient solutions (1× 50% ethanol, 2× 70% ethanol, 2× 90% ethanol, 2× 100% ethanol, 1 min each) and cleared in xylene (5 s). After air drying, the slides were mounted with DPX medium and a coverslip (VWR, Madrid, Spain). Microphotographs were acquired using a BX-61 (Olympus) microscope equipped with a DP72 camera (Olympus) and CellSens® Digital Imaging software (version 1.9).

Results

MRI analysis of patellar tendon injury

MRI sagittal images of healthy and injured patellar tendons were compared. No differences were detected in the alignment or thickness of tendon fibers along the tendon tissue. Diffuse edema affecting mainly the articular and muscular structures was observed (Fig. 3).

Macroscopic analysis of tendon injury

Clear macroscopic differences were found between healthy and injured tendons. Thus, healthy tendon showed a bright and intense white color and a strong consistency, whereas the injured tendon presented a yellowish brown color and a gelatinous presence. We observed that the peri-articular tissues, mainly the adjacent skeletal muscles, presented changes in color and consistency (Fig. 4).

Histological analysis of patellar tendon

Contralateral healthy tendon showed a uniform appearance showing compact and well aligned collagen fibers. Tenocytes showed a spindle shape and localized in parallel to the tendon fibers pattern. The structure of Hoffa's fat showed normal characteristics. In contrast, in the injured tendon a degeneration of the collagen fibre's structure was clearly observed, showing also an evident corrugated structure and empty spaces between the adjacent fiber bundles at the level of the proximal third of patellar tendon. We also observed a partial fragmentation of tendon fibers at the site of injection. Likewise, a clear disorganization of the Hoffa's fat pad tissue was detected, suggesting its affectation and disruption as a consequence of collagenase administration (Fig. 5).

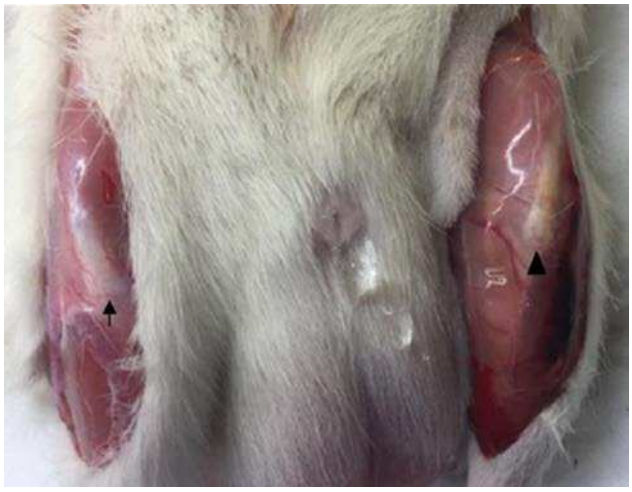

Figure 4 Macroscopic analysis. Comparison of healthy (left) and injured tendon (right). The injured tendon has a yellowish-brown color and a soft gelatinous consistency (▲).

Discussion

The main finding of the present study was the demonstration of the similarity of the structure alterations of the collagenase-treated rat patellar tendon, characterized by the disorganization of collagen fibers and adjacent structures, with respect to the patellar tendinopathy observed in the human clinics. These findings suggest that the administration of a single dose of collagenase by ultrasound-guided injection is effective to closely reproduce the structural changes in the integrity of the proximal region of patellar tendon of human patellar tendinopathy.

For the first time, we generated an animal model which mimics the tendon tissue degeneration in the most common anatomical site observed in the human patellar tendinopathy. Other animal models of tendinopathy have been designed to induce tissue degeneration in the middle third part of the patellar tendon through a surgically-created ''window''.[14–22] This mechanism of injury does not

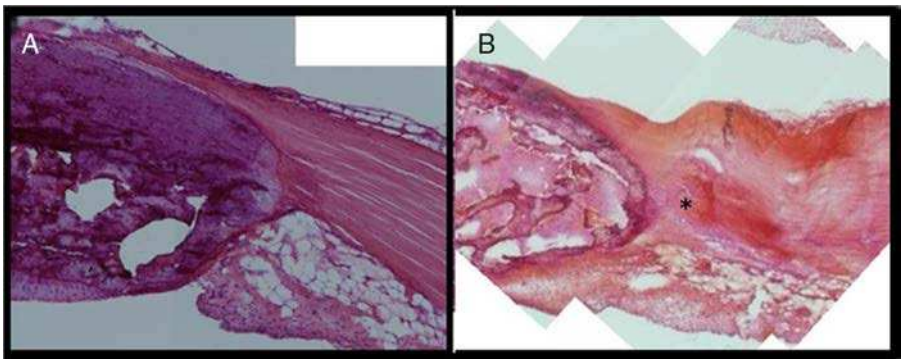

Figure 5 Comparative histological study of the healthy tendon (A) and the injured (B). The injured tendon presents a degeneration of the collagen structure at the site where the puncture was performed (*).

reproduce the lesion detected in the human clinics since the surgically-created defects are generated in tendon areas different to the most common site where the tendon pathology occurs, so we hypothesize that the tendon repair mechanisms in these models does not reproduce the pathology of the tendon subjected to repetitive work loads.[35,38] Our model is directed to imitate the degeneration of the proximal deep fibers since both the biomechanics and the relationship of the proximal patellar tendon region with the adjacent structures, mainly with the Hoffa fat pad, are important not only for the progression of tendinopathy but also in the tendon healing processes.[39,40]

Excessive tendon workload during intense physical training is considered the main cause of pathological degeneration.[41] For that reason, training has been used as a model to induce patellar tendinopathy.[36,42,43] The main disadvantage of this technique is based on the necessity to spend a long period of time for the training of the animals in order to develop the characteristic pathological changes of human patellar tendinopathy. In our opinion, it would be interesting to create a combined model of patellar tendon degeneration based on an initial collagenase-based injury by intratendinous injection in the proximal deep region followed by an intense physical training period in order to induce more rapidly the characteristic pathological degeneration of the patellar tendon. Previous data reported in Achilles tendinopathy models created by ultrasound-guided peritendinous collagenase administration suggest that the administration of collagenase may lead to tendinopathy in rats and that elastase may be also involved in the development of chronic tendinopathy.[44]

There are some limitations in our study. The first one is the small amount of animals ($N = 4$) used for the study. Second, the dose of collagenase should be optimized in order to exactly reproduce the characteristic changes in human patellar tendinopathy and prevent the collateral damage of adjacent structures to the tendon. Perucca Orfei et al.[45] generated a model in rat Achilles tendon which closely reproduced the histological changes observed in human Achilles tendinopathy. They evaluated the effect of two doses of intra-tendinous delivered collagenase (1 and 3 mg/ml). Although both doses induced disorganization of collagen fibers and increased cellularity, the higher collagenase dose treatment was able to induce a greater neovascularization and fat degeneration, being also time-dependent changes. In our study, these changes were not observed since the time elapsed from collagenase administration to tendon analysis was shorter. In addition, Perucca Orfei et al. exposed the tendon by a longitudinal incision of the skin, whereas we used a minimally invasive technique based on ultrasound-guided collagenase administration in order to induce tendinopathy in the specific proximal deep fibers region of the patellar tendon with the minimal disturbance of the structures and tissues of the knee joint. Moreover, our analysis performed by magnetic resonance demonstrates that the effect of collagenase is not localized exclusively at the tendon level but could also affect periarticular structures, a situation which has not been discussed in previous studies.

In summary, our study suggests that the ultrasound-guided administration of collagenase solution in the proximal deep region of the patellar tendon could represent a consistent model to reproduce the degeneration and changes commonly seen in human patellar tendinopathy. This new model will also allow us to develop new therapies directed to the amelioration and healing of patellar tendinopathy as a previous step to initiate clinical trials in human patients.

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Conflict of interest

The authors declare that they have no conflicts of interest directly related to the content of this article.

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References

1. Blazina ME, Kerlan RK, Jobe FW, Carter VS, Carlson GJ. Jumper's knee. Orthop Clin North Am. 1973;4:665–78.
2. Hamilton B, Purdam C. Patellar tendinosis as an adaptive process: a new hypothesis. Br J Sports Med. 2004;38:758–61.
3. Astrom M [Thesis] On the nature and etiology of chronic achilles tendinopathy. Lund, Sweden: University of Lund; 1997.
4. Kvist M [Thesis] Achilles tendon overuse injuries: a clinical and pathophysiological study in athletes. Turku, Finland: University of Turku; 1991.
5. Cook JL, Purdam CR. Is tendon pathology a continuum? A pathology model to explain the clinical presentation of load-induced tendinopathy. Br J Sports Med. 2009;43:409–16.
6. Khan KM, Cook JL, Bonar F, Harcourt P, Astrom M. Histopathology of common tendinopathies. Update and implications for clinical management. Sports Med. 1999;27:393–408.
7. Fu SC, Rolf C, Cheuk YC, Lui PP, Chan KM. Deciphering the pathogenesis of tendinopathy: a three-stages process. Sports Med Arthrosc Rehabil Ther Technol. 2010;2:30.
8. Williams IF, McCullagh GD, Goodship AE, Silver IA. Studies on the pathogenesis of equine tendinitis following collagenase injury. Res Vet Sci. 1984;36:326–38.
9. Stone D, Green C, Rao U, Aizawa H, Yamaji T, Niyibizi C, et al. Cytokine-induced tendinitis: a preliminary study in rabbits. J Orthop Res. 1999;17:168–77.
10. Soslowsky LJ, Carpenter JE, DeBano CM, Banerji I, Moalli MR. Development and use of an animal model for investigations on rotator cuff disease. J Shoulder Elbow Surg. 1996;5:383–92.
11. Warden SJ. Animal models for the study of tendinopathy. Br J Sports Med. 2007;41:232–40.
12. Lake SP, Ansorge HL, Soslowsky LJ. Animal models of tendinopathy. Disabil Rehabil. 2008;30:1530–41.
13. Warden SJ. Development and use of animal models to advance. Front Biosci (Landmark Ed). 2009;14:4588–97.
14. Lui PP, Wong OT, Lee YW. Transplantation of tendon-derived stem cells pre-treated with connective tissue

growth factor and ascorbic acid in vitro promoted better tendon repair in a patellar tendon window injury rat model. Cytotherapy. 2016;18:99–112, http://dx.doi.org/10.1016/j.jcyt.2015.10.005
15. Tan C, Lui PP, Lee YW, Wong YM. Scx-transduced tendon-derived stem cells (TDSCs) promoted better tendon repair compared to mock-transduced cells in a rat patellar tendon window injury model. PLOS ONE. 2014;9:e97453, http://dx.doi.org/10.1371/journal.pone.0097453.eCollection 2014
16. Hettrich CM, Gasinu S, Beamer BS, Stasiak M, Fox A, Birmingham P, et al. The effect of mechanical load on tendon-to-bone healing in a rat model. Am J Sports Med. 2014;42:1233–41, http://dx.doi.org/10.1177/0363546514526138
17. Xu W, Wang Y, Liu E, Sun Y, Luo Z, Xu Z, et al. Human iPSC-derived neural crest stem cells promote tendon repair in a rat patellar tendon window defect model. Tissue Eng Part A. 2013;19:2439–51, http://dx.doi.org/10.1089/ten.TEA.2012.0453
18. Ni M, Lui PP, Rui YF, Lee YW, Lee YW, Tan Q, et al. Tendon-derived stem cells (TDSCs) promote tendon repair in a rat patellar tendon window defectmodel. J Orthop Res. 2012;30:613–9, http://dx.doi.org/10.1002/jor.21559
19. Lui PP, Cheuk YC, Lee YW, Chan KMJ. Ectopic chondro-ossification and erroneous extracellular matrix deposition in a tendon windowinjury model. Orthop Res. 2012;30:37–46, http://dx.doi.org/10.1002/jor.21495
20. Ouyang HW, Goh JC, Lee EH. Viability of allogeneic bone marrow stromal cells following local delivery into patella tendon in rabbit model. Cell Transpl. 2004;13:649–57.
21. Lui PP, Chan LS, Cheuk YC, Lee YW, Chan KM. Expression of bone morphogenetic protein-2 in the chondrogenic and ossifying sites of calcific tendinopathy and traumatic tendon injury rat models. J Orthop Surg Res. 2009;4:27, http://dx.doi.org/10.1186/1749-799X-4-27
22. Chan BP, Fu S, Qin L, Lee K, Rolf CG, Chan K. Effects of basic fibroblast growth factor (bFGF) on early stages of tendon healing: a rat patellar tendon model. Acta Orthop Scand. 2000;71:513–8.
23. González JC, López C, Álvarez ME, Pérez JE, Carmona JU. Autologous leukocyte-reduced platelet-rich plasma therapy for Achilles tendinopathy induced by collagenase in a rabbit model. Sci Rep. 2016;6:19623, http://dx.doi.org/10.1038/srep19623
24. Machova Urdzikova L, Sedlacek R, Suchy T, Amemori T, Ruzicka J, Lesny P, et al. Human multipotent mesenchymal stem cells improve healing after collagenase tendon injury in the rat. Biomed Eng Online. 2014;13:42, http://dx.doi.org/10.1186/1475-925X-13-42
25. Lui PP, Lee YW, Wong YM, Zhang X, Dai K, Rolf CG. Expression of Wnt pathway mediators in metaplasic tissue in animal model and clinical samples of tendinopathy. Rheumatology. 2013 Sep;52:1609–18 [Epub 2013 June 17].
26. Dallaudière B, Lempicki M, Pesquer L, Louedec L, Preux PM, Meyer P, et al. Acceleration of tendon healing using US guided intratendinous injection of bevacizumab: first preclinical study on a murine model. Eur J Radiol. 2013;82:e823–8, http://dx.doi.org/10.1016/j.ejrad.2013.06.012
27. Lui PP, Wong Y. Higher BMP/Smad sensitivity of tendon-derived stem cells (TDSCs) isolated from the collagenase-induced tendon injury model: possible mechanism for their altered fate in vitro. BMC Musculoskelet Disord. 2013;14:248, http://dx.doi.org/10.1186/1471-2474-14-248
28. Dallaudière B, Lempicki M, Pesquer L, Louedec L, Preux PM, Meyer P, et al. Efficacy of intra-tendinous injection of platelet-rich plasma in treating tendinosis: comprehensive assessment of a rat model. Eur Radiol. 2013;23:2830–7, http://dx.doi.org/10.1007/s00330-013-2926-7
29. Rui YF, Lui PP, Wong YM, Tan Q, Chan KM. Altered fate of tendon-derived stem cells isolated from a failed tendon-healing animal model of tendinopathy. Stem Cells Dev. 2013;22:1076–85, http://dx.doi.org/10.1089/scd.2012.0555
30. Lui PP, Chan LS, Fu SC, Chan KM. Expression of sensory neuropeptides in tendon is associated with failed healing and activity-relatedtendon pain in collagenase-induced tendon injury. Am J Sports Med. 2010;38:757–64, http://dx.doi.org/10.1177/0363546509355402
31. Fu SC, Chan KM, Chan LS, Fong DT, Lui PY. The use of motion analysis to measure pain-related behaviour in a rat model of degenerative tendon injuries. J Neurosci Methods. 2009;179:309–18, http://dx.doi.org/10.1016/j.jneumeth.2009.02.011
32. Ferry ST, Afshari HM, Lee JA, Dahners LE, Weinhold PS. Effect of prostaglandin E2 injection on the structural properties of the rat patellar tendon. Sports Med Arthrosc Rehabil Ther Technol. 2012;4:2, http://dx.doi.org/10.1186/1758-2555-4-2
33. Khan MH, Li Z, Wang JH. Repeated exposure of tendon to prostaglandin-E2 leads to localized tendon degeneration. Clin J Sport Med. 2005.
34. Andarawis-Puri N, Sereysky JB, Jepsen KJ, Flatow EL. The relationships between cyclic fatigue loading, changes in initial mechanical properties, and the in vivo temporal mechanical response of the rat patellar tendon. J Biomech. 2012;45:59–65, http://dx.doi.org/10.1016/j.jbiomech.2011.10.008
35. Andarawis-Puri N, Sereysky JB, Sun HB, Jepsen KJ, Flatow EL. 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. 2012;30:1327–34, http://dx.doi.org/10.1002/jor.22059
36. Kaux JF, Drion P, Libertiaux V, Colige A, Hoffmann A, Nusgens B, et al. Eccentric training improves tendon biomechanical properties: a rat model. J Orthop Res. 2013;31:119–24.
37. Harriss DJ, Atkinson G. Ethical standards in sport and exercise science research: 2014 update. Int J Sports Med. 2013;34:1025–8.
38. Iwuagwu FC, McGrouther DA. Early cellular response in tendon injury: the effect of loading. Plast Reconstr Surg. 1998;102:2064–71.
39. Dillon EM, Erasmus PJ, Müller JH, Scheffer C, de Villiers RV. Differential forces within the proximal patellar tendon as an explanation for the characteristic lesion of patellar tendinopathy: an in vivo descriptive experimental study. Am J Sports Med. 2008;36:2119–27, http://dx.doi.org/10.1177/0363546508319311
40. Haraldsson BT, Aagaard P, Krogsgaard M, Alkjaer T, Kjaer M, Magnusson SP. Region-specific mechanical properties of the human patella tendon. J Appl Physiol (1985). 2005;98:1006–12. Epub 2004 September 24.
41. Selvanetti A, Cipolla M, Puddu G. Overuse tendon injuries: basic science and classification. Oper Tech Sports Med. 1997;5:110–7.
42. Sereysky JB, Andarawis-Puri N, Jepsen KJ, Flatow EL. Structural and mechanical effects of in vivo fatigue damage induction on murine tendon. J Orthop Res. 2012;30:965–72.
43. Fung DT, Wang VM, Andarawis-Puri N, Basta-Pljakic J, Li Y, Laudier DM, et al. Early response to tendon fatigue damage accumulation in a novel in vivo model. J Biomech. 2010;43:274–9.
44. Wu Y-T, Wu P-T, Jou I-M. Peritendinous elastase treatment induces tendon degeneration in rats: a potential model of tendinopathy in vivo. J Orthop Res. 2016;34:471–7, http://dx.doi.org/10.1002/jor.23030
45. Perucca Orfei C, Lovati AB, Viganò M, Stanco D, Bottagisio M, Di Giancamillo A, et al. Dose-related and time-dependent development of collagenase-induced tendinopathy in rats. PLOS ONE. 2016;11:e0161590, http://dx.doi.org/10.1371/journal.pone.0161590.eCollection