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REVIEW

Current concepts in the physiopathology of tendinopathies. Tissue engineering

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Abstract

Tendinopathy is a common condition that occurs while practising sport. The unequal distribution of the work load throughout the tendon causes heterogeneous ruptures in extension and distribution. These ruptures start defective repair processes that produce a degenerated tendon with a change in structure and functional response to exercise. In this article the different predisposing factors are study, along with the mechanisms of action of the chemical and cellular agents involved in the physiology of tendinopathies. The basic components (support, cells and chemical substances) that are used for tissue engineering are also analysed, as well as the current possibilities of using the basic components, the inter-relationships between them and the current level of execution. © 2010 Consell Català de l'Esport. Generalitat de Catalunya. Published by Elsevier España, S.L. All rights reserved.

PALABRAS CLAVE

Tendinopatías;
Fisiopatología;
Ingeniería tisular;
Deporte

Conceptos actuales de la fisiopatología de las tendinopatías. Ingeniería tisular

Resumen

La tendinopatía es una afectación frecuente que produce la práctica deportiva. El reparto desigual de la carga de trabajo a lo largo del tendón produce roturas heterogéneas en extensión y distribución. Estas roturas ponen en marcha procesos de reparación defectuosos que producen un tendón degenerado con alteración estructural y de la respuesta funcional al ejercicio.

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En este trabajo se estudian y analizan los distintos factores predisponentes, mecanismos de acción de los agentes químicos y celulares implicados en la fisiopatología de las tendinopatías.

Por otra parte, se analizan los componentes básicos (soporte, células y sustancias químicas) que se usan para la ingeniería tisular. Las posibilidades actuales de uso de los componentes básicos y sus interrelaciones, y el nivel actual de desarrollo.falta

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Introduction

The importance of tendon injuries in the world of sports is highlighted by the fact that tendinous involvement is a common pathology arising from sporting activity. Indeed, some authors have reported that 30-50% of all sporting injuries involve tendons¹, with up to 30% of runners, for example, having been reported to suffer from a chronic tendinopathy and 40% of racquet-sports players to suffer from tennis elbow². Degenerative involvement of the Achilles tendon, which is the most common injury in long-distance runners (56.6%), is related to the number of years the sport has been practiced and most commonly occurs in the middle part of the tendon³.

Tendinopathy of the rotator cuff is the most frequent cause of pain and dysfunction in the upper limbs and tends to appear more frequently with age⁴.

Physiopathology

From an etiopathogenic and biomechanical point of view, the load which acts on the tendon during physical exercise produces a fibrillar rupture when the mechanical traction exceeds 4% of the length at rest, with a complete rupture occurring at more than 8%. An unequal distribution of the load throughout the tendon produces ruptures that are heterogeneous in terms of their length and distribution. On the other hand, due to the different proportion of cross-links between the collagen fibres in different regions of the tendon (tendinous muscle region, mid-part, osteotendinous region), the mechanical resistance and the tendon's biochemical and structural profiles also differ^{5,6}.

These partial or fibrillar ruptures induce tendinous repair mechanisms, which involve:

- Various chemical substances, including growth factors.
- Cells such as resident tenocytes, which are involved in the balance, production and destruction of the extracellular matrix, and stem cells, which differentiate into tenocytes, adipocytes or chondrogenic or osteogenic cell lines depending, amongst other factors, on the mechanical load to which they are submitted during the repair process.
- The extracellular matrix, one of whose main components is type I collagen fibres^{6,7}.

These normal repair processes are considered defective when they produce a structurally altered degenerate

tendon. Such defective regeneration has been linked to the hypoxia level in the lesion, the presence of ischaemic damage, unequal apoptosis mediated by cytokines and inflammatory mediators, the existence of oxidative stress, the presence of local hyperthermia and an alteration to the matrix metalloproteinase (MMP) balance.

The chemical interactions in the matrix, insoluble deposits, mechanical stress, local release of cytokines and signalling molecules will all have a direct influence on tenocyte activity, cellular gene expression and the matrix enzymes.

Tenocytes play a key role in normal homeostasis, regularisation of the matrix and the pathological change which occurs during degenerative disease. Furthermore, they also appear to play a fundamental role in the incorrect production of tissue during repair of the fibrocartilage which occurs in tendinopathies⁸.

All the above processes lead to a degenerate and fibrotic tendon, thereby reducing its load-supporting ability. This therefore completes the physiopathological cycle of tendinopathies (Figure 1).

Predisposing factors

The appearance of a tendinopathy depends to a large extent on both extrinsic and intrinsic, sportsperson-related factors.

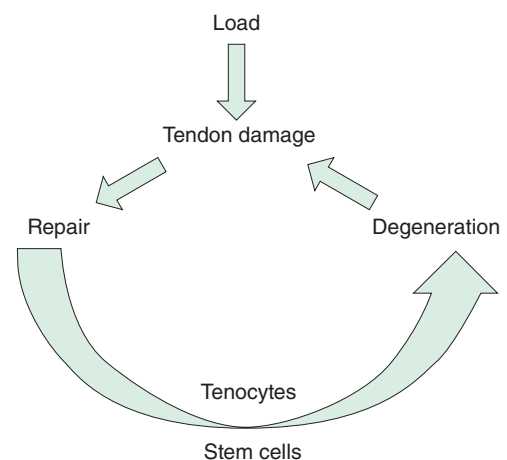


Figure 1 General scheme showing the physiopathology of tendinopathies.

Intrinsic factors: the presence of a tenascin C (TNC) gene variant and variants of the collagen V $\alpha 1$ (COL5A1) gene⁹; poor alignment of the lower limbs with varus/valgus or back knee; eversion of the ankle in runners favours the appearance of an Achilles tendinopathy^{10,11}; changes to the normal joint biomechanics, with alteration to the tendon length, changes to the muscle thickness/power ratio when taking anabolic steroids, changes to the lever arm, which result in changes to the moment of force and thus an increase in the load at one part of the tendon, have all been reported.

The factors extrinsic to the sportsperson include the following: those parameters related with the load in terms of both intensity and frequency; training; performance of a technical movement; the time allowed for the tendon to rest and recover; certain drugs (quinolones, statins) due to their interaction with metalloproteinases (MMPs) or interference with the repair mechanisms^{12,13}. MMPs themselves play a key role in tendinous degeneration¹⁴.

The highest rate of renewal of the collagen in the extracellular matrix has been associated with an increase in the expression and activity of various members of the MMP family. Thus, MMP-3 (stromelysin) is considered to be the key regulatory enzyme involved in control of matrix renewal, and a decrease in its levels could induce a change in the normal remodelling process. A study of the tendinous synovial fluid, for example, found high expression levels for MMP-1 and -3, and a molecular study of the pathology of the Achilles tendon confirmed a lack of inflammation along with marked increases in the expression of type I and type III collagen genes and an increase in the levels of versican, biglycan, perlecan and the glycoproteins laminin, SPARC and tenascin-C. MMP-3 levels were notably lower or absent in painful tendinopathy and in the event of a ruptured tendon. Drugs such as ibuprofen increase the expression of MMP-1, -2, -8, -9 and -13 without affecting the expression of type I and III collagen^{15,16}, whereas fluoroquinolones can induce tendinopathies in some cases by modulating MMP activity. Likewise, corticosteroid use also increases the risk of suffering a tendinopathy¹⁷.

Histological studies have highlighted changes to the normal distribution during healing, the lack of inflammatory cells and the existence of a poor repair response, which leads to a non-inflammatory intratendinous degeneration of the collagen fibres and thus a disorientation and thinning of these fibres, with a consequent increase in interfibrillary glucosamines, hypercellularity and disperse vascular growth. Changes to the extracellular matrix together with an increase in the expression of proteoglycans and an increase in the collagen III/I ratio have been observed in calcifying tendinopathies¹⁸.

In tendinopathies, as regards the structure of the extracellular matrix, there is: a reduction in the total collagen content, with an abnormal morphology; a reduction in the fibrillar density and a change in the alignment¹⁹, together with a higher proportion of randomly ordered type III collagen with respect to the more linear and organised type I collagen; an increase in the level of proteoglycans in those tendons undergoing a degenerative process; a build up of necrotic tissue and fibrin; an increase in the level of glycoproteins such as tenascin C; and a gradual decrease in tendon quality.

Likewise, measurements of the growth factors have shown that:

- a) Insulin Growth Factor 1 (IGF I) promotes fibroblast proliferation and migration and increases the production of collagen.
- b) Transforming Growth Factor beta (TGF beta) regulates cellular migration, cross-link proliferation and matrix remodelling²⁰.
- c) Vascular Endothelial Growth Factor (VEGF) is a powerful angiogenesis promoter. The finding that the VEGF level increases in intrinsic tenocytes suggests a role for VEGF in the angiogenesis which occurs during tendon repair. In contrast to the situation in normal tendons, an increase in the number of type 1 and 2 VEGF receptors is observed in degenerate tendons^{21,22}.
- d) Platelet Derived Growth Factor (PDGF) stimulates the production of other growth factors and plays a role in tendon remodelling.
- e) Basic Fibroblast Growth Factor (bFGF) is a strong angiogenesis stimulator and also regulates cell proliferation and migration^{23,24}. Indeed, tenocytes grow in culture when exposed to bFGF²⁵.

It has been shown that the pain caused by a tendinopathy doubles the intratendinous lactate concentration. An increase in the levels of glutamate, which is a neurotransmitter, and PGE₂, which has been linked to the onset of calcification formation, has also been observed. The level of substance P has been linked to the level of pain in tendinopathy of the cuff and elbow²⁶. Similarly, an increase in the expression of substance P and calcitonin gene related peptide (CGRP) has been observed during defective and painful tendon repair²⁷.

Histological evidence for neural growth inside the tendon and in the paratendon has also been reported in tendinopathies. This growth is regulated by pro- and anti-inflammatory mediators at the periphery of the tendon during the repair process. However, this appears to depend on the repair time, sensitive and autonomic aspects and glutamate mediators as in normal tendons nerves are only observed in the paratendon, thus reflecting normal tendon homeostasis^{27,28}.

In summary, clinical, histological and biochemical evidence for changes to tendon homeostasis during the repair and regeneration process which occurs after a post-exercise fibrillar rupture has been reported.

Tissue engineering

From a theoretical point of view, we can consider any biological tissue consisting of:

- a) A scaffold which contains the tissue support structures and is mainly composed of fibrillar substances.
- b) Cells which can possess any degree of differentiation, ranging from completely undifferentiated, such as stem cells, to highly differentiated, such as mature tenocytes.

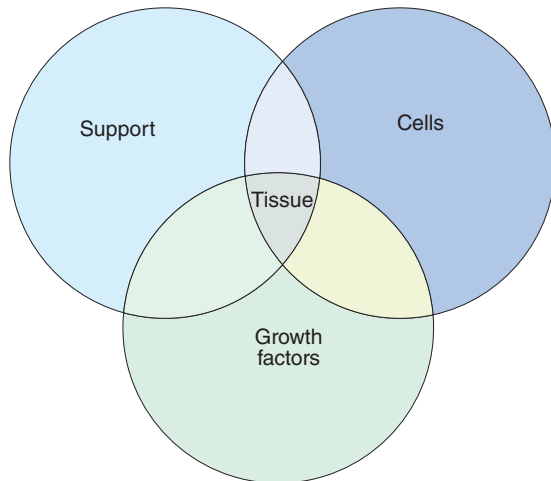


Figure 2 A graphical representation showing the components of a biological tissue.

- c) Various chemical substances, including growth factors. These chemical substances act as intracellular, intercellular and autocrine messengers and mediate the different cell responses.

Tissue engineering, which is a branch of bioengineering, aims to create or produce tissues to correct tissue defects by developing or combining one, two or all three of the above-mentioned components. Thus, a scaffold can be created to act as a host for the individual's own cells, or a scaffold with cells for implantation at the site of the tissue defect, such as a collagen matrix implant containing autologous cultivated chondrocytes (MACI; Figure 2).

The present review highlights various studies currently underway in the field of tissue engineering aimed at treating tendon injuries, many of which are still at only the animal model stage or *in vitro*.

Current possibilities

Scaffold

Biocompatible and biodegradable biomaterials are currently being used to correct bone defects²⁹, and an electrospun nanofibre structure which stimulates the differentiation of tendinous stem cells has also been created³⁰. Similarly, some studies have used calcium orthophosphate bioceramics to produce a support to correct tendinous insertion defects³¹, and others have used double-layered planar or two-dimensional polylactic acid structures³².

Combination of scaffold and cells

Fibroblasts have been embedded in a polymer gel to increase the production of fibrillar protein³³, and acellular human tendons have been implanted with cultivated tenocytes for subsequent implantation³⁴. Similarly, mesenchymal cells have been implanted in a hydrogel support³⁵, and cultivated

tenocytes have even been embedded in a pig gut scaffold³⁶.

Cells

Muscle cells subjected to a low intensity magnetic field have been implanted to regenerate tendinous muscle tissue³⁷, and cells which synthesise glycoproteins and lubricin have been implanted in the case of fracture and defect of the shoulder rotator cuff³⁸. Likewise, a technique involving cultivated and cryopreserved tenocytes has been developed³⁹, and other studies have used mesenchymal cells obtained from adipose tissue⁴⁰.

Cells and growth factors

Bone marrow mesenchymal cells have been used in conjunction with growth factors to correct tendinous defects⁴¹, whereas other studies have employed human embryonic stem cells together with foetal differentiation factor for tendon repair⁴². The possibility of implanting mesenchymal stem cells with buffered platelet-rich plasma (bPRP), which increases the proliferation and differentiation of the chondrogenic cell line, has been studied *in vitro* for use in the event of tendinous insertion defects⁴³.

Growth factors

One particular research group has developed a bioreactor to promote healing and improve the mechanical properties of tendons⁴⁴, whereas others use a soluble protein to stimulate musculoskeletal regeneration⁴⁵. Likewise, one of the most widely studied growth factors, namely basic fibroblast growth factor (bFGF), has been used to stimulate the differentiation of mesenchymal stem cells (MSCs) in the tendon⁴⁶.

Growth factors plus scaffold

Type I collagen has been combined with chondroitin 6 sulfate to promote the release of autologous mesenchymal stem cells for tendon repair⁴⁷. Likewise, bFGF has been incorporated into a biohybrid (nano and micro) fibrous scaffold to treat anterior cruciate ligament damage in the knee and tendon problems⁴⁸, and growth factor-enriched plasma has been used together with a synthetic scaffold to promote tendon cell proliferation and production *in vitro*⁴⁹. Similarly, nanofibers with gradations in mineral content have been used to mimic the tendon insertion site⁵⁰.

Cells plus growth factors plus scaffold

Autologous and homologous tissues are currently being used to perform auto- or allografts modified using the conservation technique.

In summary, various different procedures can be used to construct a living tissue. Future studies will determine which method or procedure should be used for a particular tendon injury and will establish the indications for each therapeutic option.

Conclusions

The workload applied to a tendon produces unequally distributed damage of differing severity which activates damage-regeneration and -repair mechanisms in both the extracellular and the cellular matrix. These biochemical processes reduce the tolerance of the tendon to physical exercise.

More, and more accurate, studies are therefore required in order to be able to diagnose tendinous pathologies more precisely, define therapeutic strategies and establish therapeutic protocols to restore the tendon's normal histology.

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